BULLETIN NO. 61

Proceedings of the 2025 Interbull Meeting

21-22 June 2025, Louisville, Kentucky, USA

INTERNATIONAL BULL EVALUATION SERVICE
A permanent subcommittee of the International Committee for Animal Recording
Office: Interbull Centre, Department of Animal Biosciences,
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Interbull Portfolio: Expansion of Traits

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Abstract

A total of 10 traits for three trait groups of claw health, metabolic diseases and calving were included in a new research run performed in October 2024 with the aim to expand the multiple across-country evaluation (MACE) portfolio. The traits in each trait group were as follows: digital dermatitis (dde), interdigital dermatitis (idd), interdigital hyperplasia (idh), sole hemorrhage (soh), sole ulcer (sou) and white line disease (wld) for claw health; clinical ketosis (cke), sub-clinical ketosis (sck) and milk fever (mfe) for metabolic diseases and direct gestation length (ges) for calving trait group. Although a total of 13 countries provided data for the six different breeds evaluated internationally, the research run could be performed only for four breeds (Holstein (HOL), Brown Swiss (BSW), Red Dairy Cattle (RDC) and Jersey (JER)) as only one country did provide data for Guernsey (GUE) and Simmental (SIM). The research results for across-country correlations, international EBVs and reliabilities were all promising for all evaluated traits. Gestation length was included in the Interbull May 2025 test run and subsequently in the official August 2025 routine run as the fifth trait in the MACE calving trait group alongside direct and maternal calving ease and still birth traits. The implementation of claw health and metabolic diseases traits in the MACE portfolio is currently pending a slightly higher participation rate from countries and it is therefore aimed to happen in the near future.

Key words: International evaluation, MACE, Dairy, claw health, metabolic diseases, direct gestation length

Introduction

In accordance with the Interbull new traits' pipeline introduced in 2021, countries were requested to fill in the Performance Recording, Evaluation and Publication database (PREPdb) (Interbull Centre, 2025), reporting information on any potential new traits that could be of interest for an international evaluation. The collected information included definitions of the trait, the availability of a standard International Committee of Animal Recording (ICAR) definition, the type of service, recording methods, etc. After reviewing the information, three main trait groups stood out, namely gestation length, metabolic diseases and claw health trait groups.

The results were presented during the 2024 Interbull Business Meeting, Bled, Slovenia, where it was sensed an urgency from the participating countries to have Interbull Centre performing a research run on such traits. Thus, a data call deadline for the above-mentioned trait groups was set to October 31, 2024, via the Interbull Data Exchange Area (IDEA)-new traits. By the end of the deadline, 13 countries submitted data for six breeds (Figure 1).

In total, 10 traits were included in the research run. Three and six traits were included in metabolic disease and claw health trait groups, respectively, while gestation length was assigned as the fifth trait of the calving trait group (Table 1).

Table 1. List of the new trait(s) for each trait group along with the abbreviations included in the MACE research run.

Trait group	Trait
Metabolic disease (META)	Clinical ketosis (cke)
	Sub- clinical ketosis (sck)
	Milk fever (mfe)
Claw health (CLAW)	Digital dermatitis (dde)
	Interdigital dermatitis (idd)
	Interdigital hyperplasia (idh)
	Sole hemorrhage (soh)
	Sole ulcer (sou)
	White line disease (wld)
Calving (CALV)	Gestation length (ges)

Materials and Methods

After the data submission deadline, and a preliminary screening of the data to identify the eligible breed-trait combinations, the MACE pipeline was applied. MACE started at the Interbull Centre to estimate and assess the across-country correlations, breeding values (EBVs) and reliability correlations between the national evaluations and the MACE research run

Across-country correlation estimations

The data from 13 countries and for four breeds of HOL, JER, BSW and RDC for the three new trait groups were used to estimate the across-country correlations, based on the number of common bulls between the pair of countries. The default setting for the bulls' inclusion was to have a minimum of 10 daughters in 10 herds for all the new traits. All bulls born since 1970 were included in the analysis. The Restricted Maximum Likelihood (REML) procedure was used. No subset was applied for HOL-traits, as the computing time was within the expected range due to the fact that the number of countries for the new traits was limited (the highest was eight countries for ges).

The Interbull post- processing procedure (https://interbull.org/ib/rg_procedure) was applied; all countries were assigned in one group/window of correlations defined as follows:

- Final correlations were estimated by applying a 10% percentile used for the minimum correlation
- The maximum was set to 0.99
- The median was calculated as: Median [10%;0.99]

One important step in the post-processing procedure is to assess the magnitude of the Correlation used in previous run (as there was **NO** previous run, it was set to **0.85**)

- Magnitude of changes tested was considered major for all traits meaning that the applied weight on the final correlation **was equal to 0** (equation displayed below).
- HOL correlations and common bulls were used as weighting factors for all other breeds

The windowed correlation was then calculated as follows:

$$corr_{win} = \frac{(min_{cb}*group_{medianvalue}+cb_{cou1,2}*corr_{cou1,2})}{min_{cb+}\ cb_{cou1,2}}$$

where ***cb*** is the number of common bulls between country 1 and 2.

Then, the Corr$_{win}$ were first bended to ensure that the matrix was all positive definite, and weighted against the previous correlations and the magnitude of the changes made by countries. In the next step, the results from the preceding step and the previously used correlations are combined into a weighted average to avoid large changes in correlations between consecutive test runs,

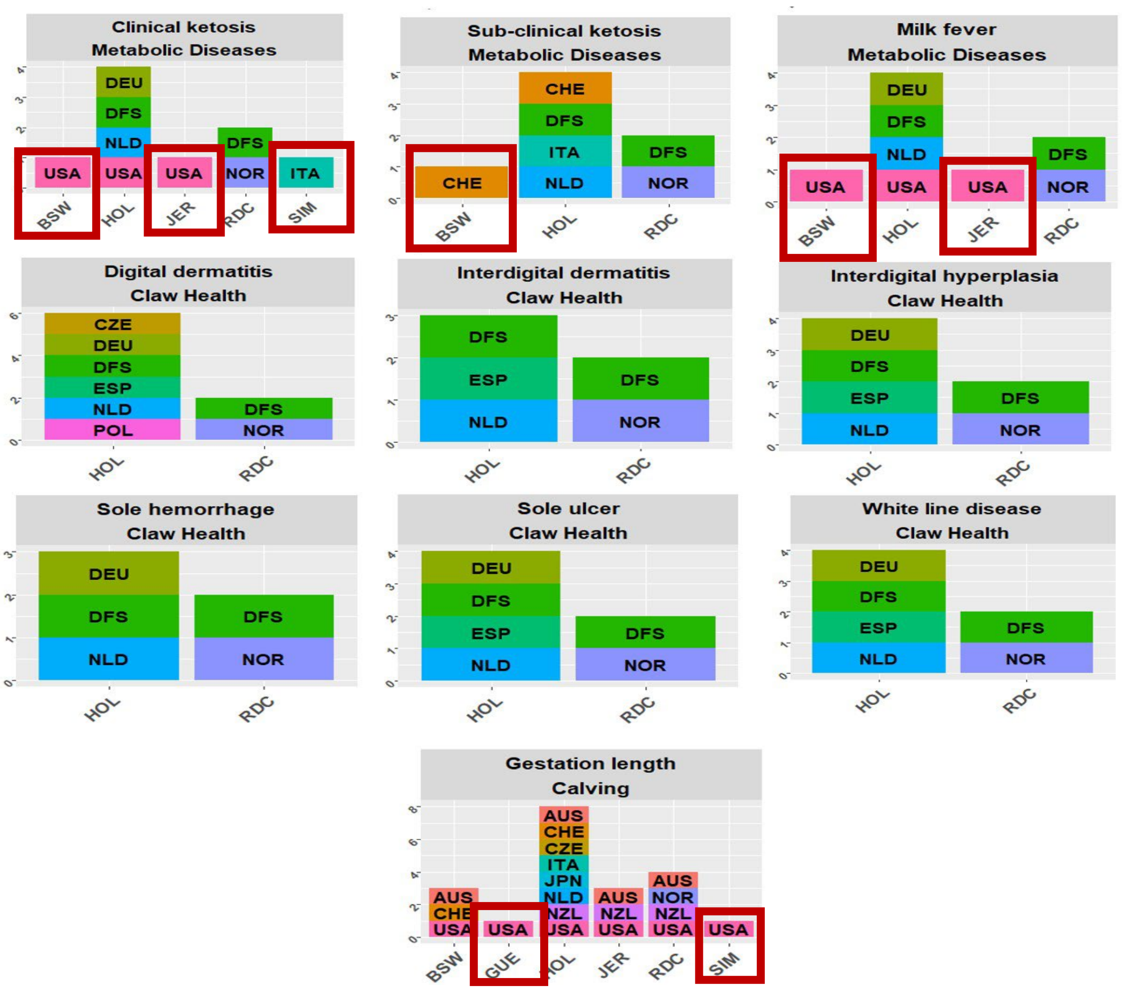

Figure 1. Breeds and countries participated for each trait and trait groups. Red ones show the breed-trait combinations which were submitted by only one country and were not included in the MACE research run.

weighted by the number of common bulls. The final correlation was calculated as follows:

$$Final_{corr} = \frac{blend_{corr} + \boldsymbol{w} * prev_{blendcorr}}{1 + \boldsymbol{w}}$$

Where $\boldsymbol{w}$ is the magnitude of changes as **0 = big change (in our case)**, 1=small and 2 = no change. If there are no changes in the national evaluations for the two countries, then the new processed correlation is not expected to deviate much from the previous one. However, if one of the countries has introduced changes in its national evaluations, it is expected that the genetic correlation between them would change as well. It is also expected that an increase in the number of common bulls would yield a more precise estimate of the genetic correlation, and in this case, less weight is given to the previous correlations. This is achieved by decreasing the weight on the previous correlations, proportionally to the increase in number of common bulls (https://interbull.org/ib/rg_procedure).

Finally, the updated (co)variance matrix is bended using the bending procedure described by (Jorjani et al., 2003).

Estimated Breeding Values (EBVs) calculation

Once all the correlation values were checked to be in the expected range the evaluation moved towards the estimation of the international EBVs. In this step, the correlation between national and international (MACE) EBVs, along with the reliability was estimated and compared.

Results and Discussion

Challenges: a) Change in directions of scales

After checking all the initial correlation estimates, some directions of scales for some countries, traits, and breeds changed as presented in Table 2, due to some results being on the negative scale.

Challenges: b) Gestation length

After the initial across-country correlation estimations for ges, two countries, ITA and CHE, had the lowest correlations with the other countries.

Table 2. Changes in direction of scale for the affected country, breed,trait with negative across-countries correlation estimations.

Breed(s)	**Trait(s)**	**Country**[1]	**Change in Direction of scale**
HOL, BSW, JER, RDC	ges	USA	T+ → T-
HOL		NLD	B+ →B-
HOL, BSW		CHE	B+ →B-
HOL		ITA	B+ →B-
RDC	cke, sck and mfe	NOR	B+ →B-

[1] United States of America (USA), the Netherlands (NLD), Switzerland (CHE), Italy (ITA), Norway (NOR)

In order to understand the root cause of the problem, Interbull Centre initiated an extensive exchange of information with the two countries involved until it came out that what those countries had submitted was *maternal* gestation length while all the other participating countries had provided the direct trait. ITA and CHE were then asked to submit direct gestation and after receiving the new data and re-estimating the across-country correlation the values became high and in line with other countries (Table 4).

Correlation estimation

All the minimum and median values applied to post-process the correlations for all breeds-traits are shown in Table 3.

Direct gestation length (ges)

Across-country correlation for direct gestation length for HOL breed is shown in Table 4. An example of number of common bulls and common ¾ sib groups only for HOL breed is presented in Table 5. Correlation estimates for RDC, JER and BSW breeds are reported in Table 6, 7 and 8, respectively. The correlation estimates ranged from 0.90 between ITA and Czech Republic (CZE) to 0.995 between USA and Japan (JPN) for the HOL breed. For the RDC breed there were four countries and the lowest and highest values were as 0.961 between NOR and New Zealand (NZL) and 0.982 between NOR and USA, respectively.

For JER breed that included three countries, correlation ranged from 0.953 to 0.983 between NZL, USA and AUS, USA respectively. The range for across-country correlation estimates for BSW breed including three countries ranged from 0.966 and 0.980 for AUS, CHE and AUS, USA accordingly.

Table 3. Minimum and Median values used for the post-processing correlation estimations for all breeds and new traits.

Breed-Trait	Min (10% percentile)	Median (10%,0.99)	Breed-Trait	Min (10% percentile)	Median (10%,0.99)
HOL-ges	0.90	0.94	HOL-idd	0.77	0.88
BSW-ges	0.96	0.98	HOL-idh	0.35	0.67
JER-ges	0.95	0.97	HOL-soh	0.59	0.79
RDC-ges	0.96	0.97	HOL-sou	0.73	0.86
HOL-cke	0.56	0.78	HOL-wld	0.63	0.81
HOL-sck	0.56	0.77	RDC-dde	0.79	0.89
HOL-mfe	0.44	0.72	RDC-idd	0.79	0.89
RDC-cke	0.54	0.77	RDC-idh	0.60	0.80
RDC-sck	0.45	0.72	RDC-soh	0.81	0.90
RDC-mfe	0.59	0.79	RDC-sou	0.91	0.95
HOL-dde	0.78	0.89	RDC-wld	0.95	0.97

Table 4. Correlation estimation for the ges in the HOL breed.

Country[1]	AUS	CHE	CZE	ITA	JPN	NLD	NZL	USA
AUS	1							
CHE	0.978	1						
CZE	0.901	0.926	1					
ITA	0.954	0.952	0.900	1				
JPN	0.986	0.982	0.901	0.956	1			
NLD	0.989	0.986	0.915	0.959	0.989	1		
NZL	0.979	0.959	0.901	0.929	0.969	0.975	1	
USA	0.985	0.981	0.902	0.962	0.995	0.993	0.974	1

[1] Australia (AUS), Switzerland (CHE), Czech Republic(CZE), Italy (ITA), Japan (JPN), the Netherlands (NLD), New Zealand (NZL), United States of America (USA)

Table 5. Number of common bulls (below diagonal) and the common 3/4 sib groups (above diagonal) for the ges and HOL breed.

Country	AUS	CHE	CZE	ITA	JPN	NLD	NZL	USA
AUS	0	320	579	854	590	823	778	1083
CHE	271	0	333	536	374	541	270	677
CZE	431	227	0	1568	917	1573	703	1846
ITA	673	444	1186	0	1325	1928	957	3103
JPN	511	284	625	935	0	1020	528	1923
NLD	691	488	1322	1396	765	0	1228	2248
NZL	716	227	520	716	397	1016	0	1206
USA	1092	580	1530	2385	1586	1725	1086	0

Table 6. Correlation estimation for the ges in the RDC breed.

RDC-ges	AUS	NOR	NZL	USA
AUS	1			
NOR	0.971	1		
NZL	0.970	0.961	1	
USA	0.979	0.982	0.966	1

Table 7. Correlation estimation for the ges in the JER breed.

JER-ges	AUS	NZL	USA
AUS	1		
NZL	0.963	1	
USA	0.983	0.953	1

Table 8. Correlation estimation for the ges in the BSW breed.

BSW-ges	AUS	CHE	USA
AUS	1		
CHE	0.966	1	
USA	0.980	0.974	1

Claw health

Across-country correlation estimations range for six claw health traits and for the two breeds of HOL and RDC are shown in Tables 9 and10. The highest value for the HOL breed was 0.928 for dde between NLD and Poland (POL) and the lowest estimate was 0.356 for idh between Spain (ESP)-NLD (Table 9).

For the RDC breed, the highest and the lowest correlations were estimated as 0.953 and 0.619 for wld and idh, respectively, between the two countries of NLD and Denmark-Finland-Sweden (DFS) (Table 10).

Table 9. Summary statistics for correlation estimation for the all six claw health traits in the HOL breed.

Traits	Breed	Min	Mean	Max
dde	HOL	0.79 (CZE,DFS)	0.86	0.928 (NLD,POL)
idd	HOL	0.772 (ESP[1]-NLD)	0.81	0.88 (ESP-DFS)
idh	HOL	0.356 (ESP-NLD)	0.59	0.881 (DEU-DFS)
soh	HOL	0.593 (DEU[1]-NLD)	0.69	0.828 (DFS-NLD)
sou	HOL	0.732 (ESP-NLD)	0.79	0.853 (ESP- DFS)
wld	HOL	0.633 (DEU –ESP)	0.71	0.8 (DEU-DFS)

[1] Spain (ESP), Germany (DEU)

Metabolic Diseases

For HOL and RDC breeds, the summary statistics for across-country correlation estimates are presented in Table 11. For the HOL breed the correlation estimations ranged between 0.444 (for mfe and Germany (DEU)-USA)) and 0.946 (sck and DFS-ITA). The highest and lowest across-country correlation estimates ranged between 0.493 and 0.605 for sck and mfe, respectively, for the RDC breed and the two countries of NLD and DFS (Table 11).

Table 10. Summary statistics for correlation estimation for the all six claw health traits in the RDC breed.

Traits	Breed	Min	Mean	Max
dde	RDC	(DFS[1]-NOR)	–	0.799
idd	RDC	(DFS-NOR)	_	0.799
idh	RDC	(DFS-NOR)	_	0.619
soh	RDC	(DFS-NOR)	_	0.821
sou	RDC	(DFS-NOR)	_	0.917
wld	RDC	(DFS-NOR)	_	0.953

[1] Denmark- Finland- Sweden (DFS)

Table 11. Summary statistics for correlation estimation for the all three metabolic diseases traits in HOL and RDC breeds.

Traits	Breed	Min	Mean	Max
cke	HOL	0.563 (DEU-USA)	0.63	0.709 (DEU-NLD)
sck	HOL	0.565 (CHE-ITA)	0.73	0.946 (DFS-ITA)
mfe	HOL	0.444 (DEU-USA)	0.55	0.695 (DEU-NLD)
Cke	RDC	NOR-DFS	-	0.558
Sck	RDC	NOR-DFS	-	0.493
mfe	RDC	NOR-DFS	-	0.605

EBVs and reliability correlation estimations; National vs International (MACE) evaluations

Direct gestation length

All the EBVs and reliability correlation estimations between the national and International (MACE) evaluations for ges and all breeds are presented in Figure 2-3. For the HOL breed, the highest EBV correlation was 1 for JPN and for the rest of the countries, the EBVs correlation were above 0.983 (Figure 2). For the RDC breed, the highest correlation is for NOR (1) and NZL (0.999) (Figure 3). For BSW and JER breeds the highest values are 0.999 for CHE and 1 for NZL, respectively (Figure 3).

Claw Health

For claw health traits, the EBV correlations between national and MACE evaluation, were quite high with the lowest being estimated for dde in the HOL breed with value of 0.9 for CZE. The highest value was 0.997 for idh and wld in NLD (Figure 4). For the RDC breed and having only two countries of NOR and DFS, the minimum value was estimated as 0.991 for wld in NOR and the highest value was estimated as 0.999 for NZL.

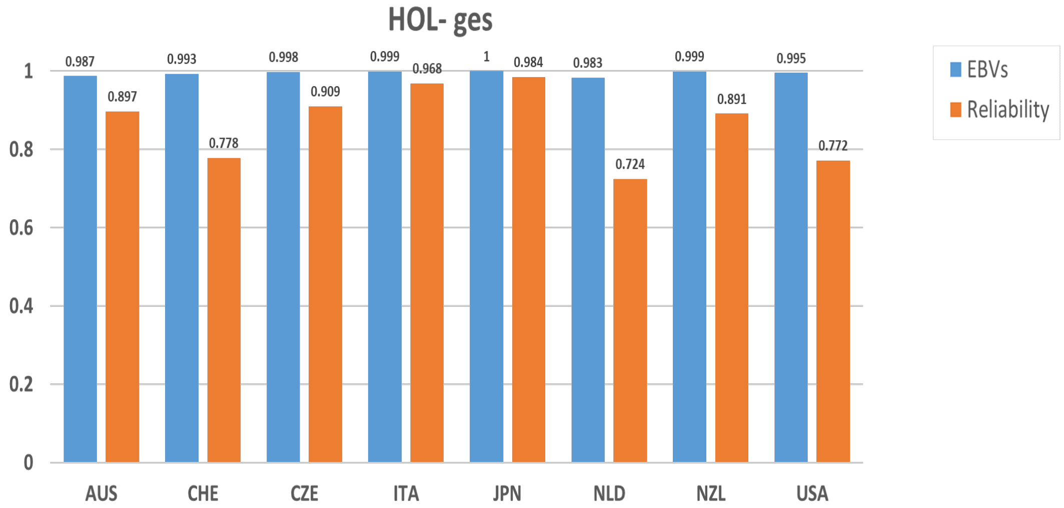

Figure 2. Correlations of EBVs and reliability estimates between national and International (MACE) evaluations for ges in the HOL breed.

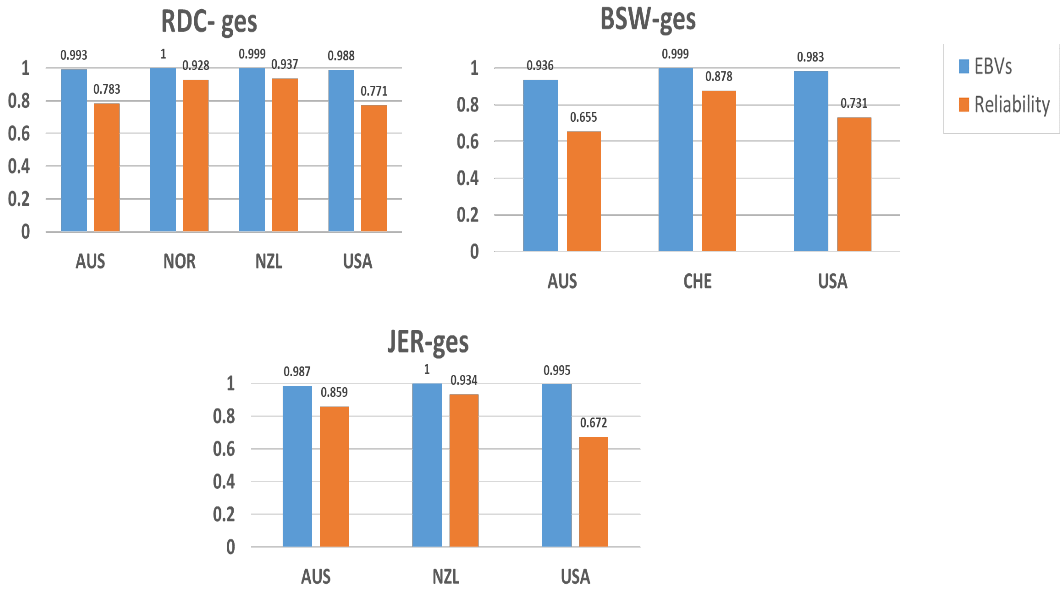

Figure 3. Correlations of EBVs and reliability estimates between national and International (MACE) evaluations for ges in RDC, BSW and JER breeds.

Metabolic disease

Table 12 presents the EBVs and Reliability correlation estimates for all three metabolic diseases traits in the HOL breed. For the HOL breed the EBVs correlations ranged from 0.924 (NLD-mfe) to 0.998 (NLD-sck; DFS-mfe). For the RDC breed and having only two countries of NOR and DFS the EBVs correlation estimations were 1 between these two countries.

May MACE test run(2505t) results- Direct gestation length (ges)

According to the new MACE service schedule, introduced in 2025, an extra MACE test run was conducted in May 2025.

Six countries participated for direct gestation length. The list of countries and breeds is presented in Figure 5. In general, the correlation estimations were similar to the research run estimates. The correlation of EBVs and reliability estimates between National and MACE May test run 2025 (2505t) evaluations was promising; Moreover, EBVs and reliability correlation estimations between May test run (2505t) and research run were also similar and promising.

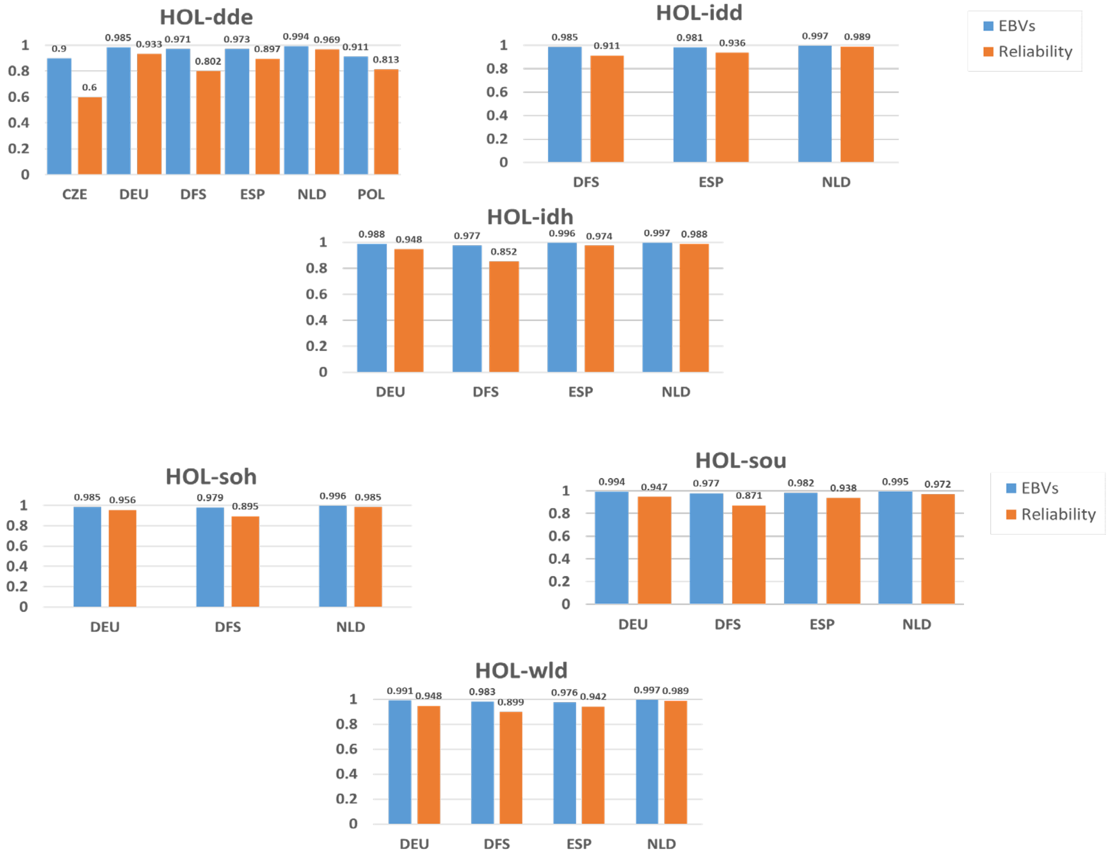

Figure 4. Correlations of EBVs and reliability estimations between national and International (MACE) evaluations for all claw health traits in the HOL breed.

Table 12. EBVs' and reliability correlation estimates between National and International (MACE) evaluations for metabolic diseases traits in the HOL breed.

		Correlation		Reliability	
Trait	**Breed**	**Min**	**Max**	**Min**	**Max**
cke	**HOL**	0.925 (NLD)	0.997 (USA)	0.868 (NLD)	0.994 (USA)
sck		0.965 (ITA)	0.998 (NLD)	0.732 (ITA)	0.986 (CHE, NLD)
mfe		0.924 (NLD)	0.998 (DFS)	0.843 (NLD)	0.996 (DFS)

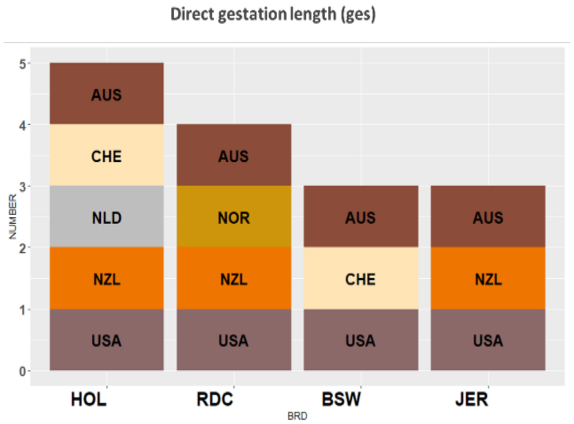
Direct gestation length (ges)

Figure 5. List of the countries and breeds, participated for "ges" in MACE May test run 2025.

Conclusion

To conclude, the new traits' MACE research run showed promising results which led to the official May test run 2025 for "ges" trait and its subsequent inclusion in the official 2025, August MACE evaluation.

Moreover, the research run results for metabolic and claw health traits have also shown the feasibility to include such trait groups in the current MACE portfolio. The offering of an official test run for those two trait groups would require a bit higher participation rate from the MACE countries/organizations and it is therefore aimed to happen in the near future. Interbull Centre will continue reviewing the new information provided by the participating countries in the PREPdb so as to timely identify any new other traits/trait groups that could be suitable for an international evaluation. In order to do that, Interbull Centre would gladly renew the invitation to all countries/organizations to continue to fill in the PREPdb "other traits" electronic form. More also can be an inclusion of more new traits and Interbull services for the new traits, such as GMACE and Intergenomics.

Acknowledgements

Interbull Centre thanks all countries for expressing interest in participating in the new traits international evaluation, the Interbull Steering Committee and the Interbull Technical Committee for the collaboration and all useful feedback.

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Genomic Evaluation for Calf Health in Canada

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Abstract

A genomic evaluation for calf health traits was developed for the Holstein breed in Canada effective August 2025. The new Calf Health index aims to increase resistance to the two most prevalent calf diseases on Canadian farms, respiratory problems (RESP) and diarrhea (DIAR). Producer-recorded respiratory problems and diarrhea health events recorded in the first 180d and 60d, respectively, of a heifer calf's life, are used in the genetic evaluation. RESP and DIAR, coded as binary traits, are used in a two-trait linear animal model considering a fixed year-season effect and random herd-year-season, animal, and residual effects for both traits. Genetic parameters were estimated by the MC EM REML method using 310 662 calf records from 1 179 herds. Heritability estimates were 0.05 for RESP and 0.04 for DIAR, with a genetic correlation of 0.53 between the traits. A Single-step genomic evaluation was implemented using the MiX99 software. A June 2025 evaluation test run had 355 355 records for RESP and 144 495 for DIAR, collected from 1 442 Canadian herds from 2007 to 2025. There were 74 013 calves with health records that were genotyped and a total of 119 715 genotyped animals in the reference population. The overall prevalence for RESP and DIAR was 19.5% and 21.1%, respectively. The Calf Health index combines genomic estimated breeding values for RESP and DIAR at equal weightings. Calf Health evaluations are published as a relative breeding value, with a mean of 100 and standard deviation of 5 for base bulls, where higher values represent greater resistance to calf health diseases. No genetic trend was observed and only weak relationships with other routinely evaluated traits were present. From a sire comparison analysis, clear differences were found when comparing high and low RBV sires in terms of daughter disease rates, highlighting the potential of the evaluation. Genetic selection for improved calf health is a valuable tool for animal welfare, lifetime animal production, and overall herd profitability.

Key words: Calf health, diarrhea, respiratory problems, single-step, genomic evaluation

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Introduction

In recent years, genetic and genomic evaluations of dairy cattle have begun to prioritize animal health. In Canada, national genomic evaluations for dairy cattle now contain various health-related traits, including mastitis resistance, metabolic diseases, hoof lesions, and fertility disorders (Jamrozik et al., 2013, Jamrozik et al., 2016, Malchiodi et al., 2020, Jamrozik et al., 2021). To date, only traits related to the mature cow have been included. However, recent studies have shown the potential for improving calf health through genetic selection, with heritabilities ranging between 0.02 to 0.24 (Gonzalez-Peña et al., 2019; Lynch et al., 2024a). The two major calf disease classes are respiratory problems (RESP) and diarrhea (DIAR). Both diseases can be caused by several pathogens which makes control of the diseases difficult on farms. Prevalence rates of RESP are typically reported between 12 and 22%, while DIAR ranged from 23 to 44% (Windeyer et al., 2014, Urie et al., 2018, USDA, 2018; Gonzalez-Peña et al., 2019). Furthermore, RESP and DIAR account for roughly 75% of preweaning mortality, highlighting the impact of calf disease on dairy farms (NAHMS, 2007, Murray, 2011).

To address this, Lactanet Canada (Guelph, ON) has developed a new genomic evaluation for both RESP and DIAR, as part of a new calf health index, officially released in August 2025 for the Holstein breed. The objectives of this study were to describe the current impact of calf diseases on Canadian farms, the methodology of the genomic evaluation, and highlight the differences in sire performance.

Materials and Methods

Data and Trait Definitions

A detailed examination of the calf health (CH) recording and traits in Canadian Holsteins can be found in Lynch et al. (2024b). Calf disease data are recorded by Canadian dairy producers on a voluntary basis since 2007. The 'healthy' herd mates were determined using herd inventory data. The two calf disease traits with sufficient records were RESP and DIAR. Only Holstein records for female calves were considered for genetic evaluations. RESP and DIAR are expressed as binary traits where 0 represents no case and 1 represents at least one disease case occurring within the defined timeframe. For RESP the first 180d of life is considered while birth to 60d is used for DIAR. To ensure accurate and continuous data recording within individual herds, at least 2 recorded cases for a given disease were required within the dataset, with a minimum of 4 months between the 1st and the last record for a trait. Also, a minimum disease frequency of 1% within a herd-birth year was required.

Using the above criteria, data used in the June 2025 evaluation included 378 587 total records, with 355 355 and 144 495 for RESP and DIAR, respectively. A total of 121 263 records had values for both traits.

Model

The model is a two-trait linear animal model for RESP and DIAR. The same model is used for both traits, considering the fixed effect of year-season and random effects of herd-year-season (**HYS**), animal additive genetic, and residual. In matrix notation, the model can be written as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1h} + \mathbf{Z_2a} + \mathbf{e},$$

where y is a vector of observations (binary RESP and DIAR traits), **b** is a vector of the fixed effect, **h** is a vector of HYS effects, **a** is a vector of animal additive genetic effects, **e** is a vector of residuals, and **X**, $\mathbf{Z_1}$, and $\mathbf{Z_2}$, are the respective incidence matrices. Random effects were assumed to be normally distributed, with means equal to zero.

Model assumptions are that: $v(\mathbf{h}) = \mathbf{I} \otimes \mathbf{HYS}$, **I** is an identity matrix and **HYS** is the covariance (2x2) matrix for HYS effects, $v(\mathbf{a}) = \mathbf{H} \otimes \mathbf{G}$, **H** is a combined pedigree-genotype relationship matrix, **G** is the additive genetic covariance, $v(\mathbf{e}) = \mathbf{R}$, **R** 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 2024 data extract including 310 662 records from 1 179 herds. The edits described above were also applied to the genetic parameter estimation dataset. 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 two-trait component-wise Single-Step GTABLUP method (Mantysaari et al., 2017) was implemented at Lactanet Canada using MiX99 and related software (MiX99 Development Team, 2017), with the assumption that 80% of the total genetic variance was explained by SNP effects. The June 2025 data included 119 715 genotyped animals, of which 74 013 were genotyped females with phenotypes and 8 570 were genotyped sires with phenotyped daughters. Animals are genotyped either with 50K SNP panel or a low-density panel and imputed to 50K using F-Impute (Sargolzaei al., 2014). 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 (Luke, Finland). Animal model reliabilities are calculated with the EDC and reliability software of Sullivan (2023).

Relative Breeding Values

The CH index combines the two individual RESP and DIAR traits at equal weighting. The index and the individual traits are published. The evaluations are expressed as Relative Breeding Values (**RBV**) with a mean of 100 and SD of 5 for base bulls that for April 2025 are those born 2010-2019 and with an 'official' status. A higher RBV value means a greater resistance to calf health diseases. Sire evaluations are defined as 'official' for RESP and DIAR when they have at least 20 phenotyped daughters from 5 herds for the respective trait and a minimum reliability of 70%. Sires are official for CH when they are official for both contributing traits.

Sire Comparison Validation

To investigate the difference in performance of top and bottom performing sires, a random cross-validation study was conducted. For each trait, official sires with at least 30 phenotyped daughters were included in the analysis. Randomly half of each sire's phenotyped daughters had their phenotype changed to missing, while the remaining half were used to predict sires' RBV. Sires were then ranked according to their RBV. The daughters with their phenotype set to missing for the evaluation were used to determine the sires' daughter disease rate for both the calf diseases, therefore acting as an independent sample. Sires with an RBV greater than 110 and lower than 90 were then compared based on their percentage of disease daughters for each calf disease. This process was repeated five times and averaged across iterations to get an accurate representation of sire performance across different sample groups.

Results and Discussion

Incidence Rates

Incidence rates across years for RESP and DIAR are shown in Figure 1. On average, the incidence rates for RESP and DIAR were 19.5% and 21.1%, respectively, which were similar to values reported in the literature (Lynch et al. 2024a). Greater fluctuation has been seen in DIAR incidence rates, whereas RESP has remained relatively stable. This fluctuation may be due to several factors, including changes in herds reporting information and quality of reporting over time.

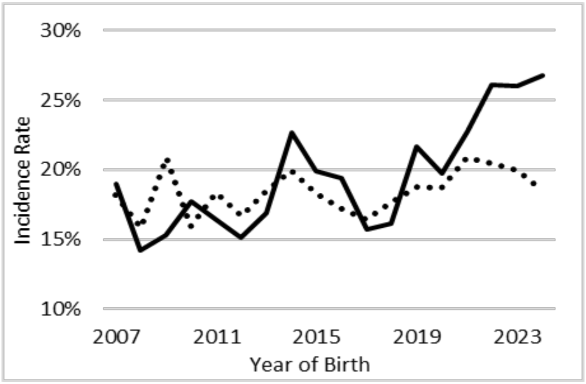

Figure 1: Incidence rates across years for Respiratory Problems (dotted line) and Diarrhea (solid line).

Genetic Parameters

Heritability and genetic and phenotypic correlation estimates for RESP and DIAR are shown in Table 1. The heritabilities for RESP and DIAR were 0.054 and 0.044, respectively. Estimates were similar to those reported in the literature for calf disease traits and other health related traits in the Canadian Holstein population (Lynch et al. 2024a, Jamrozik et al., 2013, Jamrozik et al., 2016, Malchiodi et al., 2017, Jamrozik et al., 2021).

Table 1: Heritabilities with standard error in parentheses, genetic correlations (above diagonal), and phenotypic correlations (below) diagonal for Respiratory Problems (RESP) and Diarrhea (DIAR).

	RESP	DIAR
RESP	0.054 (0.010)	0.53
DIAR	0.13	0.044 (0.013)

Genomic Evaluations

In the June 2025 preliminary evaluation run there were 1 393 Holstein sires with an official CH evaluation. The RBV for CH evaluation ranged from 78 to 114 for this group and averaged 100. The average reliability was 87% and ranged from 72 to 99% for official sires. The average reliability of genotyped, young Holstein bulls without daughter records that were identified as being controlled by an AI organization (N=3 744) was 70%.

Proof correlations were estimated between CH index, RESP and DIAR and other routinely evaluated traits in Canada using 937 Holstein sires born since 2010 with an official LPI and CH index. The proof correlation between RESP and DIAR was 0.39, which is similar but slightly lower than the genetic correlation estimate. This highlights some difference in the two traits and that the CH index is useful to make effective progress in both traits, since the proof correlation for RESP and DIAR with CH is 0.83 and 0.84, respectively. For other routinely evaluated traits, little to no correlations were found. No proof correlations above |0.20| were found between CH and RESP for any other trait currently being evaluated. For DIAR, the only proof correlations stronger than 0.20 were for Calving Ability (-0.20) and Calving Ease of daughters at first and later calvings (-0.21 and -0.20, respectively). Direct selection on the CH index is therefore important to make progress in the calf health traits analyzed.

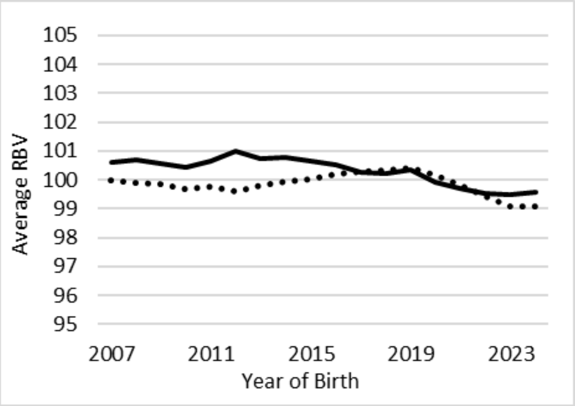

Figure 2: Genetic trend for Holstein females with records for Respiratory Problems (dotted line) Diarrhea (solid line).

The genetic trend for CH in females with records in the evaluation is shown in Figure 2. The genetic trend has been relatively flat since the onset of trait recording. Since there is little to no relationship with other traits under selection in the Canadian Holstein population, this is expected.

Sire Comparison

On average, daughters born to sires with an RBV less than 90 were 1.8 times more likely to exhibit DIAR compared to daughters born to sires with an RBV greater than 110, while for RESP they were 1.3 times more likely. These differences help highlight the difference in sire performance and show the potential of the evaluation to help improve the health of young dairy calves. These results are in line with a similar approach conducted by Lynch et al. (2024b).

Conclusions

Genetic improvement of Calf Health is highly valuable as it impacts replacement loss, lifetime performance, animal welfare, and overall profitability. The first genomic evaluations for the Calf Health index and the contributing traits, DIAR and RESP, were published in August 2025 by Lactanet for the Canadian Holstein breed. The introduction of the CH index in the Canadian national selection index LPI within its Health and Welfare subindex is scheduled for April 2026.

Acknowledgements

All Canadian dairy producers recording health data are gratefully acknowledged. Research leading to the implementation of genetic evaluation for calf health traits in Canada was part of the Resilient Dairy Genome Project (RDGP). We gratefully acknowledge all funding and support for the Resilient Dairy Genome Project from the organizations listed at http://www.resilientdairy.ca/funders-and-partners/, as administered by Genome Canada, Genome Alberta, Ontario Genomics, Genome Quebec, and Genome British Columbia.

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Genomic predictions for dairy calf health traits

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Abstract

Healthy calves are important to the productivity and welfare of dairy herds. They are potential herd replacements as well as a source of livestock trading income. Further, healthy calves are important to the continuous improvement of animal welfare that is valued by farmers and consumers. In our dataset of ~20,000 calves with health records, the prevalence of stillbirth, preweaning mortality and scours was 4%, 2% and 6% respectively suggesting that there are opportunities to improve calf health. The aim of this study was to estimate variance components for novel calf traits and gather the perspectives of farmers about the relative importance of these traits. Univariate linear models that included a genomic relationship matrix were used to estimate variance components for stillbirth, preweaning mortality, scours, respiratory disease and calf vitality where heritability (h^2) estimates ranged from 1% to 11% depending on the trait. Calf vitality is a new, subjectively-scored trait where farmers describe calves on a scale from A (vigorous) to E (dead). The models included herd-year-season, sex, parity group and calving ease as fixed effects and these were found to be significant for most breed and trait combinations. Our survey found that calf traits were valued by farmers similarly to cow survival. They preferred new traits to be published separately, rather than in multi-trait indexes. As genetic variation in several calf health traits was measured and the value to farmers has been tested, we conclude that there is an opportunity to introduce new traits into routine evaluations that target genetic gain for calf health.

Key words: Calf health, stillbirth, vitality, breeding values

Introduction

Healthy calves are an important part of a dairy herd's natural cycle. Heifer calves become replacements that enable a herd to sustain or grow its size. Replacement heifers are costly to rear. In fact, Boulton et al. (2017) reported that it takes 1.5 lactations to repay the costs associated with the heifer rearing period. As morbidity increases, the costs associated with extra labour and treatments are expected to rise. As mortality rises, the total costs are spread over fewer surviving animals. There are economic, productivity and welfare benefits arising from healthier calves.

Compared to cow health traits, the genetic contribution to improved calf health and lower mortality is a relatively new area of research but it is a logical progression to the successful genetic improvement of traits like udder health (Abdelsayed et al., 2017) and fertility (Ooi et al., 2023) in cows and the number of stillborn calves (Cole et al., 2007).

This paper reports variance components for calf health traits and industry perspectives about trait expression and their relative importance for breeding purposes.

Materials and Methods

Health records for 19,824 calves were collected from ~50 Australian dairy herds as previously described by the authors (Axford et al., 2025a). Calf health events and deaths were coded as binary traits for analysis as 0 or 100 for each trait, where sick or dead was coded as 0 and healthy was 100. The traits were stillbirth (SB) (dead at birth or shortly thereafter), pre-weaning mortality (PWM) (born alive but died before weaning, estimated to be day 84), Health (presence of any health event), Scours (presence of any diarrhea event), Resp (presence of any respiratory disease). Vitality was a subjectively scored trait with 5 levels where A was a vigorous calf, B was a good calf, C was an average calf, D was a dull calf that lacked vigour and E was a dead calf.

Genetic parameters were estimated using univariate linear animal models that included a genomic relationship matrix (GRM) and fixed effects in ASReml 4.2 (Gilmour et al., 2022). The fixed effects were calving ease (CE) with 3 levels (no assistance, slight assistance and moderate/high assistance), dam parity at calving where parity was divided into 2 levels (parity 1 and parity 2+), sex of the calf and Herd Year Season (HYS) where season was divided into 2 levels (1 is January-June, and 2 is July-December). Calving ease was dropped in Jersey models because there were few cases of dystocia recorded in the dataset. Due to data limitations, direct-effect models were used. Mating data for dams and further detailed calf phenotypes were unavailable so gestation length, birth weight and colostrum were not included in the model. Animals were used in the EBV predictions if they were genotyped, sire by a recorded, AI sire and there was a minimum of 5 records in the HYS.

The general form of the model used to estimate variance components and genomic breeding values for each trait was as follows:

$$y=Xb+Zu+e$$

where **y** is the vector of the phenotypic records for each trait (SB, PWM, Health, Scours, Resp, Vitality); **b** is the vector of the fixed effects including HYS, parity group, CE for Holstein only, and sex; **u** is the vector of the random additive genetic effect and **e** is the vector of random residual effects; **X** and **Z** are design matrices that relate phenotypes to their corresponding fixed effects (**b**) and random additive genetic effects (**u**). It is assumed that

$$var(u)=GRM\sigma_u^2,\ var(e)=I\sigma_e^2$$

where σ_u^2 is the additive genetic variance, σ_e^2 is the residual variance, and **I** is an identity matrix.

This model was expanded to include two traits and was used to check the genetic correlation between calf traits of interest. Further, to test the relationship with cow traits, approximate genetic correlations were calculated using Peason correlations and then adjusted for reliabilities as we described earlier (Axford et al., 2025a).

The reliability of prediction for all traits was calculated using the standard errors of EBV, as follows:

$$reliability=1-\frac{PEV_i}{\sigma_u^2}$$

where, PEVi is the prediction error variance (squared error of the EBVi for animal i in the pedigree) and σ_u^2 is the estimated genetic variance in the prediction model.

To gather the perspectives of farmers and service providers about the importance of calf traits in breeding programs, an online survey was conducted between October 2023 and June 2024 using SurveyMonkey (https://uk.surveymonkey.com/). Respondents were asked about their business and herd demographics, calf record keeping, trait preferences and opinions about the expression of genetic traits. A total of 109 responses were received, of which 66% were farmers with further demographic details available in Axford et al. (2025b).

Results & Discussion

Disease prevalence

Table 1 reports the prevalence of morbidity and mortality for Holstein and Jersey calves. The prevalence of SB was lower (4% compared to almost 7%) to our earlier Australian study of a larger national dataset (Axford et al., 2024) and the prevalence of PWM was similar (~2%). This dataset was more recent (calves born 2020-2023) and involved farmers that agreed to participate in this calf research who may prioritise calf health and recording which could explain the lower mortality rate. As expected, scours was the most commonly recorded disease, followed by respiratory disease. Few cases of other health events were recorded, for example miscellaneous (96 cases), deformities (26 cases), and pink eye (20 cases). Stillbirth explained five times more deaths than scours and respiratory disease combined, suggesting that this was a major calf welfare issue on participating dairy farms.

The novel trait of calf vitality had fewer records (n=3,651) as roughly half of the herds routinely recorded this trait. Twenty-one percent of recorded calves were scored as A - “vigorous”, 28% B - “good”, 26% C – “average”, 6% D – “dull”, and 19% E – “dead”. Many herds (40%) only recorded vitality scores for dead calves which explains the high percentage of “E” scores in the dataset.

Table 1: Across herd prevalence of morbidity and mortality in Holstein and Jersey calves, expressed as a percent.

	Holstein (n=11,182) Overall mean % (SE)	Jersey (n=949) Overall mean % (SE)
Pre-Weaning Mortality	2.0 (0.1)	2.7 (0.5)
Respiratory disease (lived and died)	0.4 (0.1)	0.1 (0.1)
Respiratory disease (died)	0.1 (0.0)	0.0 (0.0)
Scours (lived and died)	5.9 (0.2)	4.8 (0.7)
Scours (died)	1.0 (0.1)	1.5 (0.4)
Stillbirth	4.1 (0.2)	4.8 (0.7)

Genetic parameters

After editing to include animals with a genotype, recorded AI sire and at least 5 records per HYS, there were 7,504-10,513 records for Scours, SB and PWM. HYS were removed if the Vitality records included only calves scored as E – “dead” leaving 1,693 Vitality records remaining. The heritability ranged between 1-11% depending on the trait. Either low disease prevalence, smaller sample size or a combination of the two meant that variance components for Jersey cattle could not be estimated.

Table 2: Genetic variance (VarG), phenotypic variance (VarP), and heritability (h^2) estimates for calf health traits in Holstein cattle from univariate linear models.

Trait	VarG (SE)	VarP (SE)	h^2 (SE)
Holstein			
PWM	0.43 (0.38)	76.91 (1.07)	0.01 (0.01)
Scours	17.48 (4.05)	390.25 (6.44)	0.04 (0.01)
Stillbirth	5.68 (2.07)	230.86 (3.59)	0.03 (0.01)
Vitality	44.66 (15.16)	392.69 (13.82)	0.11 (0.04)

Stillbirth, as the major cause of early life mortality, had a heritability estimate of 4% (for the direct effect). At least in Holstein cattle, selecting for calving ease contributes to lower stillbirth rates as the genetic correlation is favourable (0.7 between stillbirth direct and calving ease, Axford et al., 2024). However, other significant effects, such as parity, are uncontrollable as there will always be heifer calvings. Therefore, adding stillbirth into sire selection protocols is an important step in improving calf welfare.

Calf scours was the most prevalent disease reported in this study and others (Neupane et al., 2021, Urie et al., 2018). As is common for health traits, including mastitis (Abdelsayed et al., 2017), the proportion of variance explained by genetics is low. In our case, the heritability of scours was 4% and this was similar to a recent Canadian study (4-6%, Lynch et al., 2024). The mean sire EBV for scours was 0.05

(±1.86 SD) as shown in Figure 1 and mean reliability was 0.27 (±0.11 SD).

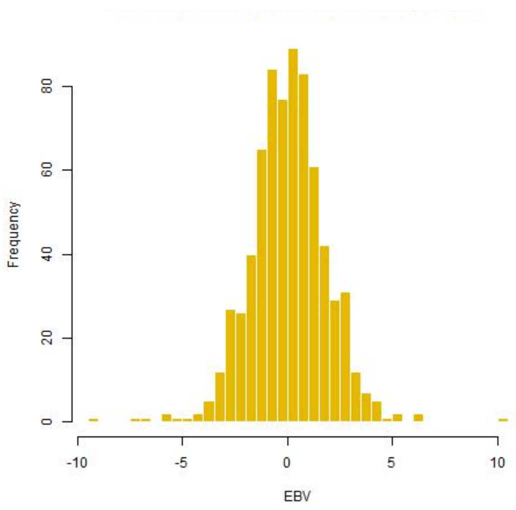

Figure 1. Distribution of EBV for scours in Holstein sires

Scours is a major contributor to PWM. About half of the calves that were born alive but died before weaning were recorded as having died from scours in this study. Interestingly, the genetic correlation between the two was only 0.18. PWM had a very low heritability estimate of only 1% in this study, which is lower than the 9% reported by Zhang et al. (2022) with a similar model. Despite significant efforts to obtain a dataset of sufficient size, traits with low prevalence are especially challenging in genetic analysis and emphasise the need for more systematic approaches to data recording, at scale, such as automatic milk feeders and calf health sensors.

Vitality was an experimental trait that is thought to reflect both health and behavioural characteristics and the interaction between the two. For example, a calf that is highly motivated to drink more milk may achieve higher intakes that promote good health. Despite having the least records, the heritability estimate for vitality was highest (11%). It is likely that the multiple levels partially explain the higher heritability compared to the remaining calf traits. There was a moderate relationship between vitality and scours (genetic correlation 0.46) suggesting that the trait of vitality is capturing different information compared to scours alone. There were no significant genetic correlations between vitality and traits of the cow, such as Cow Survival, Likeability (another subjectively scored trait) and the Balanced Performance Index (BPI, national breeding index).

Survey

From this research, it is clear that genetic variation from calf health traits can be measured and EBVs could be incorporated into routine genetic evaluation. However, the availability of EBVs is not enough to instigate practice-change on-farm. As genetic selection decisions are the domain of farmers, their opinions are important. On a preference scale of 1-5 where 5 was most important, the mean score ranged between 3.5 (±1.1) for heifer survival from weaning to first calving and 3.8 (±1.1) for calf health, as shown in Figure 2. These scores were lower than production traits but higher than scores for new traits such as feed saved and heat tolerance. Calf trait scores were similar to traits that are included in BPI, such as cow survival, mastitis and type traits.

With regard to the expression of calf traits, respondents preferred that calf traits were presented so that higher ABVs reflect healthier calves (88%) and preferred traits to be presented separately rather than in a multi-trait index. We suggest that the preference for single trait presentation is related to the desire for transparency when new traits are first released.

Conclusions

The genetic selection for calf traits is a natural extension to the highly successful genetic improvement of traits affecting the productive life of cows. Like many other health traits, the calf traits we studied are characterised by low heritability yet are highly valued by farmers. There are opportunities to improve the welfare of calves and lower the costs associated with rearing replacements by adding calf health traits to routine genetic evaluations.

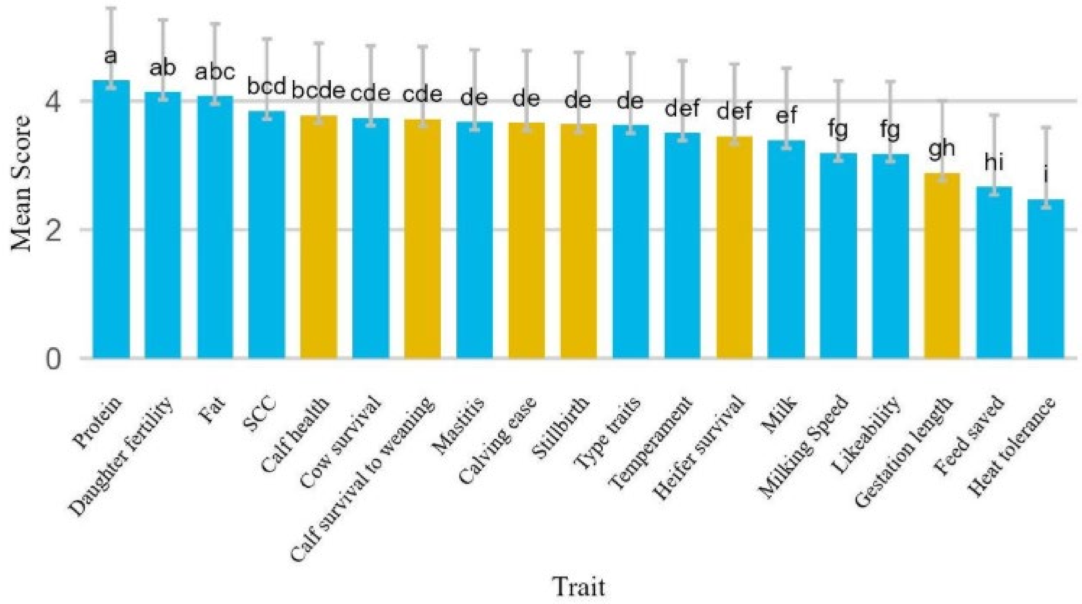

Figure 2. Weighted mean scores (bars) and standard error (whiskers) for calf (yellow) and cow (blue) trait preferences where 5 is most important and 1 is least important. Bars with no common letters identify scores that are significantly different ($p<0.05$).

Acknowledgments

This research was funded by DairyBio, an initiative of Dairy Australia, Agriculture Victoria and The Gardiner Foundation and contributed to a PhD project at La Trobe University. The authors thank DataGene, herd recording centres and dairy farmers for recording data that was used in this research and Zoetis for supporting the genotyping of dead calves.

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Update of genetic parameters and inclusion of Swedish and Finnish β-hydroxybutyrate and acetone measurements to the Nordic General Health evaluation model

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Abstract

The Nordic (Denmark, Finland, Sweden) General Health (GH) evaluation model was introduced in 2008 and significantly revised between 2017 and 2019. The current GH index includes reproductive disorders, feet and leg disorders, clinical ketosis, and other metabolic diseases recorded as veterinary treatments. Acetone and β-hydroxybutyrate (BHB) measurements from milk mid-infrared (MIR) spectra are used as correlated traits in mixed model equations and supplied primarily from Danish herds. Although the collection of Swedish acetone and BHB measurements began in 2018, this data has not yet been incorporated into official evaluations. Furthermore, new Finnish measurements are available only for BHB and predicted from MIR using a different equation than those used in Denmark and Sweden.

Swedish BHB and acetone, along with Finnish BHB data, were integrated into the official Nordic evaluation pipeline. Genetic correlations between Finnish and Swedish/Danish BHB were estimated at around 0.8. Genetic parameters were newly estimated for Holstein, Red Dairy Cattle (RDC), and Jersey breeds. The largest changes in heritability and genetic correlations between clinical and subclinical ketosis were observed for RDC and Jersey. Correspondingly, the largest changes in breeding values were observed for RDC and JER Nordic AI bulls. The updated model is planned to be implemented in November 2025.

Key words: BHB, Nordic Dairy Cattle Evaluation, Metabolic disorders, Variance component

Introduction

Metabolic disorders are commonly observed conditions in high-yielding dairy cattle, affecting health, productivity, and economics of a herd. Ketosis and subclinical ketosis are considered as the most prevalent metabolic disorders in dairy cows (Eduardo and Barrientos-Blanco, 2024). Joint selection for resistance to clinical ketosis in Denmark, Finland, and Sweden (DFS) began in 2008 when the first General Health (GH) model and index were developed (Johansson et al., 2008). In 2017, the GH model was enhanced by inclusion of milk biomarker traits β-hydroxybutyrate (BHB) and acetone (ACE) to perform selection against subclinical ketosis (Rius-Villarasa et al., 2018).

Biomarkers show a strong correlation with clinical ketosis and other metabolic diseases. In DFS, BHB and ACE indexes are not summands of the GH index and only used as correlated traits in the mix model equation. Phenotypes are measured using mid-infrared (MIR) milk spectra during the first 60 days of lactation. In the current workflow, biomarker data primary originates from Denmark; data from Finland is a fixed data set collected between 2016 and 2019, and there is no data from Sweden. Due to limited data for Red Dairy Cattle (RDC) and

Jersey (JER) available for variance component (VC) estimation, parameters were derived from the Holstein breed (HOL).

The aims of the current project were: 1) inclusion of Swedish BHB and ACE, and Finnish BHB data into the Nordic GH model; 2) estimation of genetic parameters for BHB and ACE in RDC and JER; 3) investigation of differences of the BHB and ACE phenotypes between countries.

Materials and Methods

Data

The Nordic GH model includes five treatment traits and two biomarker traits (Table 1). Treatment data was previously described in Rius-Villarasa et al. (2018) and include veterinary records for early and late reproductive disorders (ERP and LRP), feet and legs disorders (FL), clinical ketosis (KET), and other metabolic diseases (OMB). Biomarker data (BHB and ACE) were based on mid-infrared spectra analyzed by Foss MilkoScan (MilkoScan FT+, Foss Electric A/S, Hillerød, Denmark). For Denmark (DNK) and Sweden (SWE) BHB and ACE concentrations in milk were predicted from the spectra using the Foss prediction equation. For Finland (FIN) BHB concentrations in blood were predicted from the spectra using the approach shown in Kostensalo et al. (2023). Subset of FIN cows (n=134,232) had BHB phenotypes predicted using both the Foss and Kostensalo et al. (2023); however, BHB from the Foss predictions were not used in the estimation of genetic parameters or breeding values.

Table 1: Number of cows with records by trait and breed.

Trait*	HOL	RDC	JER
erp1	7802789	4366832	668042
lrp1	7712623	4326174	655709
fl1	7712623	4326174	655709
ket1	7712623	4326174	655709
omb1	7712623	4326174	655709
bhb1	1422300	181433	252271
ace1	1422300	181433	252271
erp2	5531607	3166152	462345
lrp2	5463053	3132902	454798
fl2	5463053	3132902	454798
ket2	5463053	3132902	454798
omb2	5463053	3132902	454798
bhb2	1121718	147311	200307
ace2	1121718	147311	200307
erp3	3488821	1992511	307564
lrp3	3435200	1966113	301615
fl3	3435200	1966113	301615
ket3	3435200	1966113	301615
omb3	3435200	1966113	301615
bhb3	789610	101109	142485
ace3	789610	101109	142485

*erp – early reproductive disorder; lrp – late reproductive disorder; fl – feet and legs disorders; ket – clinical ketosis; omb – other metabolic disease; bhb – β-hydroxybutyrate; ace – acetone.

Data for variance component estimation

For the estimation of genetic parameters subsets with highly reliable bulls were used. The subsets were defined as bulls with ≥ 50 daughters in ≥ 25 herds for HOL, and ≥ 25 daughters in ≥ 10 herds for RDC and JER. For BHB and ACE, records from DNK collected from 2019 onwards were used. Genetic parameters for KET, OMB, BHB, and ACE were calculated using breed x country specific combinations. Data from DNK were used for HOL and JER breeds, while SWE data were used for RDC (Table 2).

Table 2: Number of sires and cows by trait and breed used for 12 trait VCE.

Trait*	HOL		RDC		JER	
	sires	cows**	sires	cows**	sires	cows**
bhb1	546	493	295	69	162	109
ace1	546	493	295	69	162	109
ket1	528	414	295	65	153	89
omb1	528	414	295	65	153	89
bhb2	515	307	283	35	150	64
ace2	515	307	283	35	150	64
ket2	459	229	277	32	130	46
omb2	459	229	277	32	130	46
bhb3	447	169	238	17	126	33
ace3	447	169	238	17	126	33
ket3	383	116	230	14	110	21
omb3	383	116	230	14	110	21

*erp – early reproductive disorder; lrp – late reproductive disorder; fl – feet and legs disorders; ket – clinical ketosis; omb – other metabolic disease; bhb – β-hydroxybutyrate; ace – acetone
**Cows are in thousands (*1000)

Mixed model equation

The following mixed model equations were applied for veterinary treatments and biomarkers:

$$Y_{ijkl} = CHY_i + CCA_j + CYM_k + u_l + e_{ijkl}$$

and

$$Y_{ijklm} = CHY_i + CCA_j + CYM_k + L1_{ijklm} + L2_{ijklm} + u_l + pe_m + e_{ijklm}$$

Where, Y_{ijkl} and Y_{ijklm} were individual observations for veterinary treatments and metabolic biomarkers, respectively. Fixed effects were: CHY_i – the country-herd-year, CCA_j – the country-calving-age; CYM_k – the country-heard-month. $L1_{ijklm}$ and $L2_{ijklm}$ were regression for lactation stage modelled as a first and second order Legendre polynomial. Random effects pe_m and e_{ijkl} were permanent environment and residual effects, respectively. Random effect u_l is the animal effect in the breeding value estimation and sire effect in the variance component estimation.

Variance component estimation

Variance component estimation was performed using sire model and DMUv6. r.5.6 software (Madsen and Jensen, 2024) in two setups: 1) to estimate genetic correlations between countries and establish adjustment factors (κ) for BHB and ACE where each country-parity combination was treated as a separate trait; 2) to estimate new parameters for routine use, a 12-trait model including BHB, ACE, KET, and OMB in parities 1-3 was used. Newly estimated parameters for BHB and ACE (6x6 block) were used to replace current routine estimates. Newly estimated covariances between BHB[ACE] and OMB, and between BHB[ACE] and KET were used to update routine covariances. The matrix bending procedure (Jorjani et al., 2003) was applied to make the 21 trait (co)variance matrix positive definite.

Biomarkers data adjustment

Prior to breeding value evaluation BHB and ACE records were multiplied by country-parity specific κ calculated as:

$$\kappa = \sqrt{4\sigma^2_{s\_des}/4\sigma^2_{s\_obser}}$$

Where $\sigma^2_{s\_des}$ is sire genetic variance in desired breed-country-parity strata, and $\sigma^2_{s\_obser}$ is sire genetic variance in observed breed-country-parity strata. For HOL and JER the variance was estimated based on DNK data, and for RDC variance was estimated based on SWE data.

Because sire variance was not possible to estimate for FIN and SWE JER, variance ($\sigma^2_{s\_CNTR}$) was approximated by parity using following formula:

$$\sigma^2_{s\_CNTR} = \left(\frac{\sigma^2_{s\_DNK}}{\sigma^2_{p\_DNK}}\right) * \sigma^2_{p\_CNTR},$$

where $\sigma^2_{s\_DNK}$ is a sire variance in DNK data, $\sigma^2_{p\_DNK}$ is a phenotypic (data) variance in DNK data, and $\sigma^2_{p\_CNTR}$ is a phenotypic (data) variance in FIN or SWE.

Results & Discussion

Biomarker data difference

Inter-country genetic correlations of BHB and ACE in parity 1 for HOL and RDC are shown in Table 3. The average genetic correlation between FIN and the other countries was 0.84. A similar correlation (0.81) was obtained from regression of FIN cows phenotypes predicted using the Foss vs Kostensalo et al. (2023) approach (Figure 1).

Table 3: Inter-country genetic correlations for BHB and ACE in parity 1

Countries	HOL	RDC
	BHB	
SWE x DNK	0.95	0.97
FIN x SWE	0.86	0.88
FIN x DNK	0.84	0.79
	ACE*	
SWE x DNK	0.95	0.97

*No ACE data available for Finland

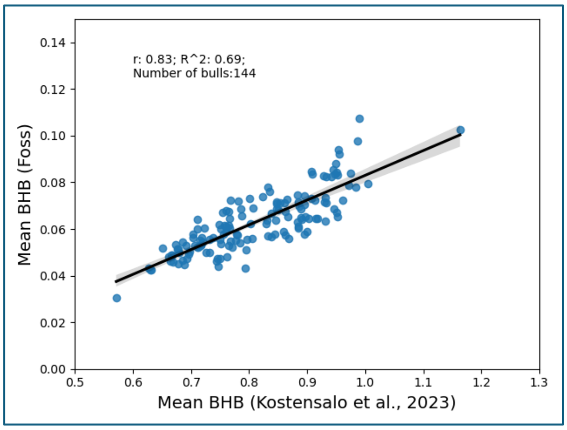

Figure 1. Scatter Plot and Linear Regression of first parity mean BHB phenotypes of daughters of HOL bulls with >50 daughters.

The correlation between milk and blood BHB concentration was expectedly high. However, FIN BHB trait is similar, but not identical to DNK and SWE. The scale difference in the phenotype was handled by data adjustment factor (κ). As BHB and ACE are not summand of the GH index it was decided to use blended-origin trait.

Genetic parameters

Newly estimated heritability, genetic and permanent environment correlations for BHB and ACE are presented in Table 4. For HOL the heritability was slightly lower for BHB (average: 0.04) and slightly higher (average: 0.01) for ACE compared to current routine estimates. Changes in correlations ranged from -0.10 to 0.14. For RDC the heritability increased for both BHB and ACE, with a range from 0.01 to 0.06. Overall heritability in RDC was estimated to be higher than in other breeds. Genetic correlation between BHB and ACE, and between parities, also increased by 0.01-0.33. For JER the heritability increased for both BHB and ACE, with an average of 0.03. Genetic and permanent environment correlations increased in the range of 0.02 to 0.37. Overall changes for HOL were concluded to be limited, but sufficient and positively accepted for RDC and JER.

Genetic correlations between biomarkers and metabolic traits are shown in Table 5. For HOL, the largest difference compared to currently used parameters were observed for the BHB x KET and ACE x OMB combinations, with decreases of 0.16 and 0.17, respectively. For JER, a slight increase (0.13) was observed for the BHB x OMB correlation. A large decrease in correlation was observed in RDC for BHB x OMB and ACE x OMB pairs - 0.15 and 0.36, respectively. Although the decrease in correlation is unfavorable, it is important to note that the RDC parameters were previously approximated from HOL, whereas they are now directly estimated.

Table 4. Heritability, genetic and permanent environment correlation in BHB and ACE parity 1-3.

Traits	BHB1	ACE1	BHB2	ACE2	BHB3	ACE3
			Holstein			
BHB1	**0.08***	0.88	0.85	0.72	0.78	0.66
ACE1	0.53	**0.05**	0.73	0.80	0.67	0.70
BHB2	0.00	0.00	**0.09**	0.89	0.95	0.86
ACE2	0.00	0.00	0.60	**0.05**	0.87	0.94
BHB3	0.00	0.00	0.00	0.00	**0.08**	0.92
ACE3	0.00	0.00	0.00	0.00	0.62	**0.04**
			Red Dairy Cattle			
Traits	BHB1	ACE1	BHB2	ACE2	BHB3	ACE3
BHB1	**0.14**	0.89	0.93	0.81	0.90	0.85
ACE1	0.68	**0.09**	0.83	0.89	0.77	0.87
BHB2	0.00	0.00	**0.14**	0.90	0.93	0.88
ACE2	0.00	0.00	0.71	**0.09**	0.80	0.91
BHB3	0.00	0.00	0.00	0.00	**0.13**	0.92
ACE3	0.00	0.00	0.00	0.00	0.72	**0.09**
			Jersey			
Traits	BHB1	ACE1	BHB2	ACE2	BHB3	ACE3
BHB1	**0.08**	0.93	0.86	0.62	0.81	0.54
ACE1	0.56	**0.05**	0.82	0.74	0.79	0.69
BHB2	0.00	0.00	**0.09**	0.84	0.94	0.73

ACE2	0.00	0.00	0.60	**0.04**	0.81	0.87
BHB3	0.00	0.00	0.00	0.00	**0.08**	0.84
ACE3	0.00	0.00	0.00	0.00	0.65	**0.04**

*Diagonal – heritability, upper triangle – genetic correlation, lower triangle – permanent environment correlation.

Table 5. Genetic correlations between biomarker (BHB and ACE) and metabolic traits (KET) in first lactation.

Breed	HOL		RDC		JER	
Traits	BHB	ACE	BHB	ACE	BHB	ACE
KET	0.56	0.59	0.63	0.63	0.64	0.67
OMB	0.47	0.48	0.31	0.26	0.46	0.47

Breeding value.

Impact of the new genetic parameters and added biomarker data on EBVs is presented through; 1) Correlation of the current and new sub-indexes (Figure 2, 4 & 6), and 2) Reranking of sub-indexes (Figure 3, 5 & 7) in Nordic AI bulls with ≥20 daughters.

Correlation was high (>0.95) and reranking was limited for HOL bulls born after 2015. The largest changes were observed for BHB, ACE, KET, and OMB traits. For this group of traits, low correlation (<0.95) and a mean reranking > 1 index unit were observed for bulls born 2010-2014, due to the absence of BHB and ACE data before 2018 (historic Danish BHB and ACE data was discarded from the data).

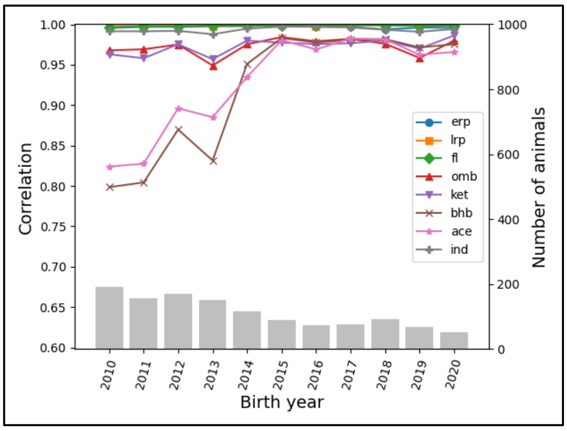

Figure 2. Correlation of current and new GH (sub)indexes in HOL AI bulls with ≥ 20 daughters.

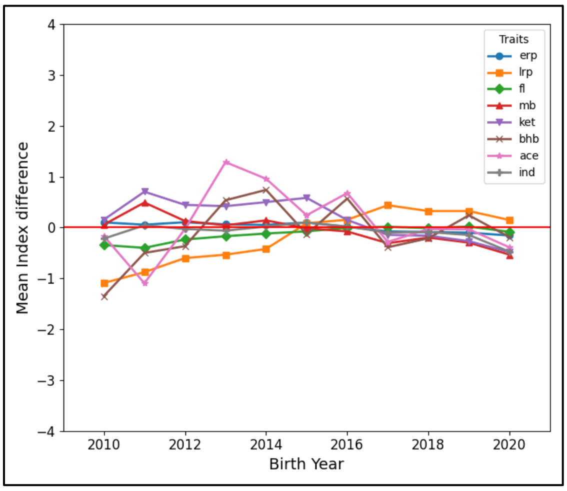

Figure 3. Mean index difference between current and new GH model in HOL AI bulls with ≥ 20 daughters.

In RDC, the correlation was below 0.90 for BHB and ACE across all year classes. For OMB and KET, the correlation declined from 2016 onwards which can be explained by a gap in SWE treatment data recording during 2023-2025 and absence of ACE records in FIN data. The main sources of RDC data were SWE and FIN. High reranking (> 1 index unit) was observed in the BHB and ACE subindexes of bulls born before 2015.

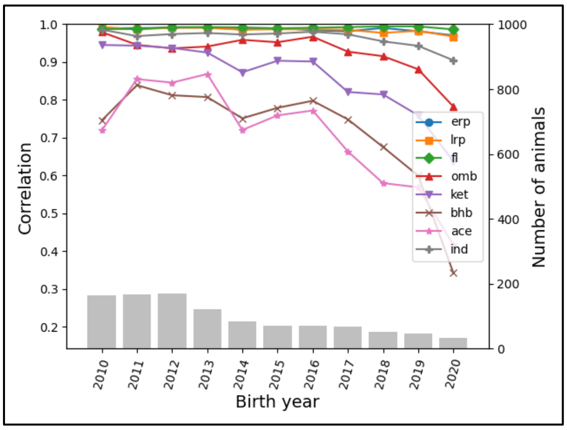

Figure 4. Correlation of current and new GH (sub)indexes in RDC AI bulls with ≥ 20 daughters.

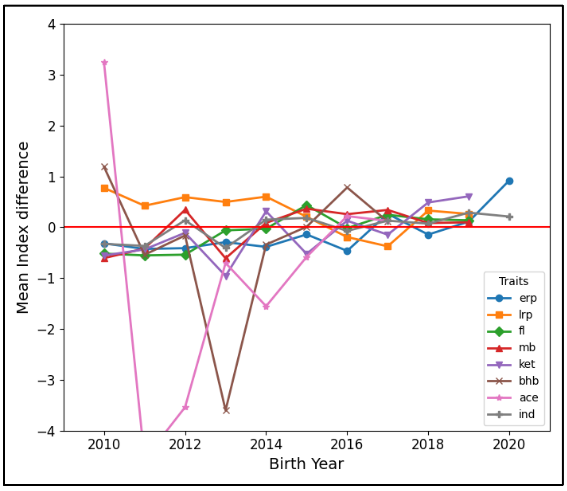

Figure 5. Mean index difference between current and new GH model in RDC AI bulls with ≥ 20 daughters.

For JER, the correlation between current and new models for BHB and ACE was below 0.9 in bulls born between 2010-2013. The correlation below 0.95 was also observed for KET. The highest reranking was seen in BHB, ACE, OMB, KET, and LRP. Presumably, the LRP trait was influenced through its correlation with OMB and KET. The maximum observed mean reranking was 2 index units.

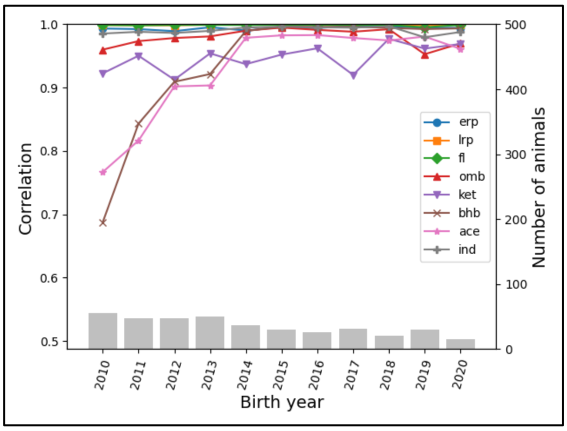

Figure 6. Correlation of current and new GH (sub)indexes in JER AI bulls with ≥ 20 daughters.

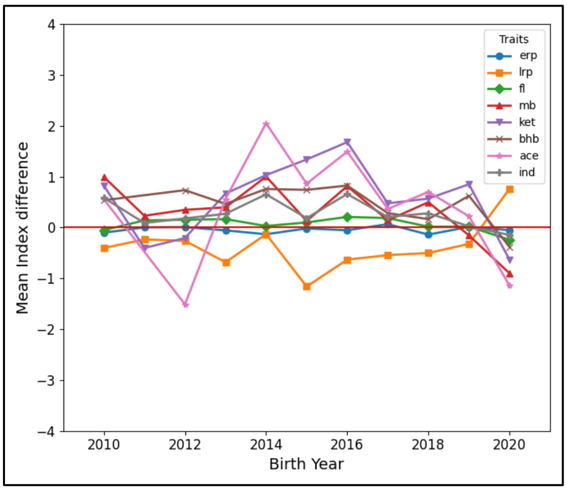

Figure 7. Mean index difference between current and new GH model in JER AI bulls with ≥ 20 daughters.

Correlation and reranking patterns were as expected. Stepwise changes to the model showed that the largest effect was caused by new genetic parameters and changes in data structure.

Conclusions

The inclusion of Swedish BHB and ACE records has expanded data set, especially for RDC breed. Genetic parameters for RDC and JER were based on direct estimation rather than approximation and better align with the data. The new parameters caused greater reranking in RDC and JER than in HOL. Aceton records for RDC will be predominantly based on SWE data, as FIN has stopped ACE recording.

The updated GH index will be available to Nordic farmers starting in November 2025. The presence of national data is expected to increase trust from Swedish farmers towards the GH index.

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Genetic evaluation of twinning rate in Italian Holstein

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Abstract

Twinning in Holstein cattle is unfavorably linked to calving difficulties, abortions, milk production and reduced calf survival. The twinning rate in the Italian Holstein population is 2.2% and appears relatively stable over time; however, this figure does not account for early abortions and thus it is underestimated. This study aimed to establish a routine genetic evaluation of twinning rate in the aforementioned population. The phenotype of interest was the type of calving (0 = singleton; 1 = twins). The statistical model employed included, as random, herd-year of conception, permanent environmental effect and the cow's additive genetic effect. Fixed effects comprised year-season of conception, herd, synchronization protocol (classified into three categories: yes, partial or no), days in milk class and parity-age-year of conception. The dataset included 11,329,160 records after filtering, with age at calving restricted to 18-77 months and parity limited to maximum three. Only fixed effects levels comprising at least 100 observations were retained. The minimum number of contemporaries was set to 10. Data editing was loop-based to simultaneously meet all the described restrictions. Genetic parameters were estimated on a sample of 500 randomly selected herds using THRGIBBS1F90 software. Posterior mean of heritability for twinning rate was 1%. To validate the accuracy and stability of the predictions a genomic validation was conducted. Genomic validation yielded a dispersion of 0.94 and validation reliability of 0.18. This study has laid the foundation for the implementation of a routine genetic evaluation of twinning rate in the Italian Holstein breed.

Key words: twinning, dairy cattle, genomic selection, codominance, model validation, genetic parameters

Introduction

Twinning in cattle can result from either monozygotic or dizygotic embryos. Monozygotic (identical) twins arise when a single fertilized egg splits into two embryos, whereas dizygotic (fraternal) twins originate from the fertilization of two separate eggs by two different sperm cells. In the Holstein breed, the vast majority of twins are dizygotic, originating from multiple ovulations and separate fertilizations. The most likely cause of twinning is the codominance of multiple dominant follicles, which is often associated with low progesterone (P4) concentrations during the follicular waves (Martins et al, 2018). This hormonal environment alters the secretion patterns of follicle-stimulating hormone (FSH), promoting the simultaneous development of multiple dominant follicles.

Multiple pregnancies increase the risk of reproductive and metabolic disorders, stillbirth, freemartinism and early pregnancy loss: the estimated cost of a twin pregnancy ranges from \$59 to \$161 (Cabrera et al, 2021).

In the light of the above, the aim of this study was to implement twinning rate (TWI) into the routine genetic and genomic evaluation system for Italian Holsteins.

Materials and Methods

Data editing

Data after edits consisted of 12M records of calving events from 1987 onwards. Maximum parity order was set to three and the following ranges of age at calving within parity order were defined: 18-41 moths (parity one), 30-59 (parity two) and 42-77 (parity three). The days in milk (DIM) range at conception was 21-305 while the gestation length range was 240-315 days. The minimum number of contemporaries for herd-year of conception was 10. The minimum number of observations per level of fixed effect was 100. All the criteria were assured to be met with a loop-based approach. The classification of synchronization protocol application was derived from the weekly distribution of inseminations in each herd during each period, considering 2010 as lower cutoff year. When more than 75% of inseminations occurred on a specific day of the week, it was assumed that synchronization was applied to the entire herd. If the percentage ranged between 35% and 75%, partial application of the protocol was assumed. Below 35%, it was considered that no synchronization was applied. Farm sizes and time trends within farms were taken into account to enhance classification accuracy. Each group corresponds to different synchronization strategies and, consequently, to different expected effects on the phenotype. Pedigree was traced back to 3 generations.

Statistical model

A single trait repeatability linear animal model was used, with twinning (TWI) as dependent variable. Following a previous study on the Italian Holstein breed (Katende et al, 2025), it was possible to apply a linear model with the cow as the additive genetic effect. The choice of a linear model did not result in significant differences compared to the theoretically more appropriate threshold model. The direct effect, that of the sire, was found to be negligible and was therefore not included in the model.

The model was the following:

$$\mathrm{TWI}_{ijklmnop} = hy_i + S_j{*}Y_k + \mathrm{H_l} + DIM_l + AGEC\_PAR_{\mathrm{m}}{*}Y_k + \mathrm{SYNC_n} + a_o + pe_{\mathrm{o}} + e_{ijklmnop}$$

with $\mathrm{TWI}_{ijklmnop}$ as the pth binary (singleton/twin) phenotypic observation of twin calving. Fixed effects were $S_j{*}Y_k$ as the crossed effect of season j by year k of conception, $\mathrm{H_l}$ as herd of conception, DIM_l as the lth days in milk at conception class (10 classes of 30 days), $AGEC\_PAR_{\mathrm{m}}{*}Y_k$ as the mth age at calving by parity class (9 classes: 3 age at calving classes for every parity class) by year k. Random effects were hy_i as the ith contemporary group for herd-year of conception, a_o as the additive genetic effect of the oth cow, pe_{o} as the permanent environmental effect of the oth cow and $e_{ijklmnop}$ as the residual of observation p.

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 635,026 animals (500 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, 2015). Genomic evaluation was performed with a SNPBLUP model using GS3 software (Legarra et al, 2011). For estimated deregressed proofs (EDPs), the method from Degano et al (2016) was applied. A conventional quality control was applied to SNP data. For the imputation process, PedImpute software was used (Nicolazzi et al, 2013). Approximate genetic correlations were calculated as Pearson correlation coefficients between genomic estimated breeding values (GEBVs) of 3,200 heifers born in 2025.

Conventional, genomic and phenotypic 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 2504 run) and one reduced (with a 4-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.

Parameters considered were the dispersion coefficient and the reliability of the linear regression model (validation reliability). To assess the validity of predictions at phenotype level, a sample of 1,168 females with phenotypes in the full run but not in the reduced run was selected. Their DGVs from the reduced run were standardized, and the average phenotype was evaluated for each standardized category.

Results & Discussion

The average frequency of twinning across the entire dataset was 2.2%, in agreement with the literature (Kirkpatrick et al., 2025). The twinning rate in the Italian Holstein population is estimated at 2.2% It has remained fairly constant over time; however, this value is likely underestimated as it does not include early abortions. The posterior mean of heritability was estimated at 0.01 (posterior standard deviation: 0.001), which falls within the ranges reported in the literature (Kirkpatrick et al., 2025; Lett et al., 2018; Katende et al., 2025; Hüneke et al., 2025).

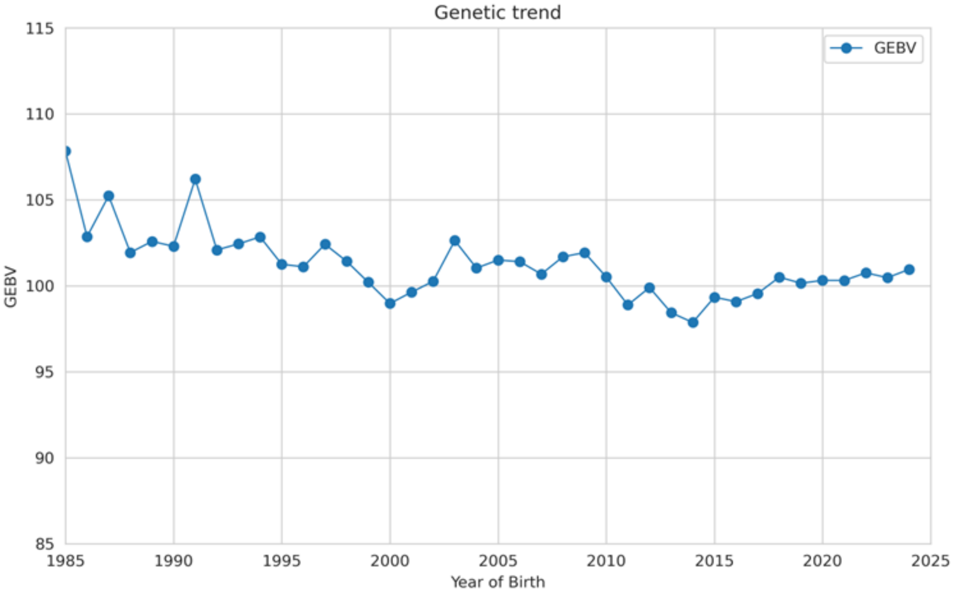

Figure 1. Bulls' genetic trend by birth year. GEBV=average GEBV.

This result confirms the potential for selection on this trait, despite the challenges posed by its low magnitude. GEBVs are expressed with a mean of 100 and a standard deviation of 5, with values above 100 referring to animals with lower genetic potential for twinning (and therefore considered favorable). The trend of GEBVs, reported in Figure 1, displayed a decline until the last 10 years, during which a reversal was observed, likely linked to improvements in traits related to female fertility. A similar trend was identified by Kirkpatrick (2025). Indeed, genetic selection for reducing twinning rate in dairy cattle may be desirable, provided it does not lead to undesirable correlated responses in other economically important traits. Twin births in dairy cattle are typically associated with negative outcomes, such as impaired reproductive performance and decreased calf survival.

The approximate genetic correlations are depicted in Figure 2: for all traits related to fertility and milk quality, correlations were low but favorable. Regarding milk yield, a slight negative correlation was detected, probably due to a faster metabolism leading to more rapid degradation of P4.

The genomic validation resulted in a dispersion coefficient of 0.94, indicating a negligible overestimation of the DGV and confirming the accuracy and stability of both the statistical model and the genetic evaluation procedure. The validation reliability resulted 0.18.

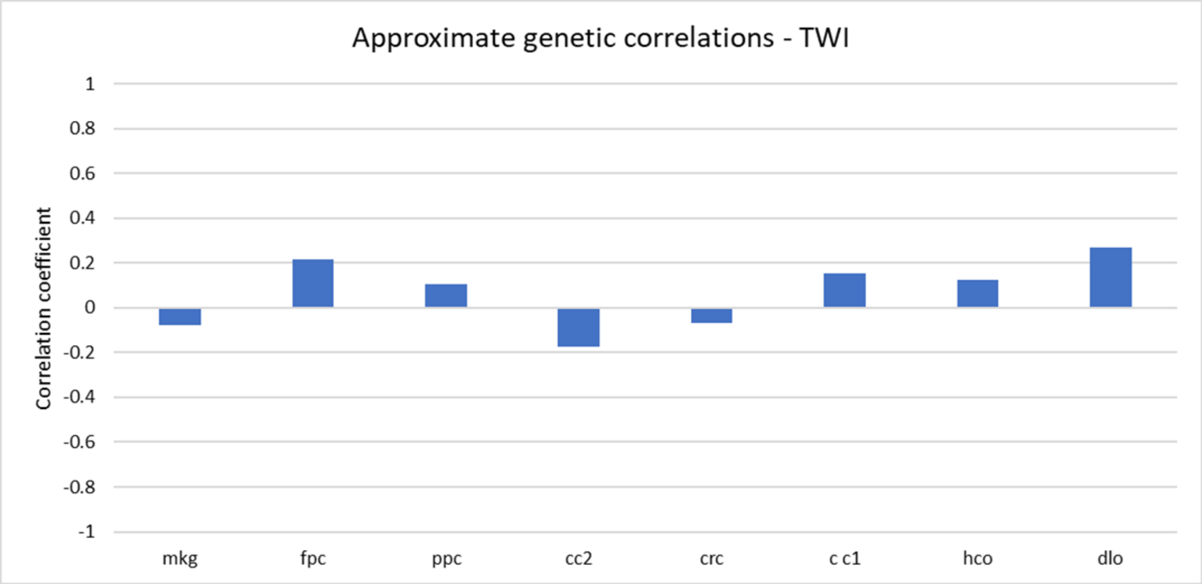

Figure 2. Approximate genetic correlations for TWI. Mkg=milk yield, fpc=fat percentage, ppc=protein percentage, cc2=interval first-last insemination cows, crc=cow recycling, cc1=conception rate cows, hco=conception rate heifers, dlo=direct longevity.

Phenotypic validation, represented in Figure 3, confirms the accuracy of the predictions in relation to future phenotypes. Indeed, when considering only the groups with a sufficient number of observations to ensure a reliable mean (ranging from -2 to +1 standard deviations from the mean), we observe that higher genetic indices are associated with more favorable future phenotypes.

Conclusions

In conclusion, this study increased the knowledge about the genetic aspects of TWI in the Italian Holstein population and revealed the possibility to genetically improve the breed for this trait. Moreover, it confirmed the stability of the applied model and its ability to predict future phenotypes through genomic evaluation. Moreover, it confirmed the stability of the applied model and the ability of genomic evaluation to predict future phenotypes, thus providing solid decision-making support for the selection of breeding animals both at the AI center and farm level. A routine genetic evaluation of TWI will be soon implemented in Italy for the Italian Holstein breed.

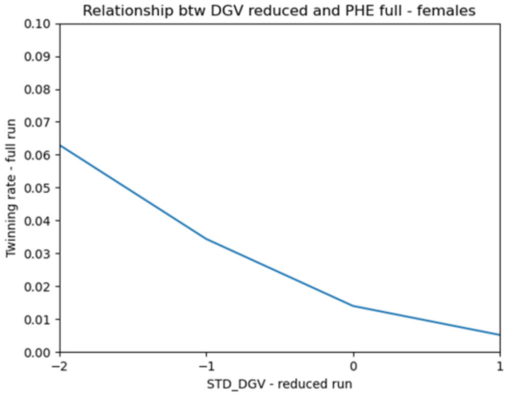

Figure 3. Average full-run phenotype (Twinning rate - full run) and standardized DGV (STD_DGV - reduced run) for females lacking phenotypes in the reduced run.

Acknowledgments

This study was supported by “Latteco2 project, sottomisura 10.2 of the PSRN-Biodiversity 2020–2023” (MIPAAF. D.M. no. 465907 del 24/09/2021, project unique code J12C21004080005).

The authors would like to thank Dr. Massimiliano Lanteri (DVM) and Dr. Stefano Allodi (DVM) for their valuable support and collaboration.

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Genetic correlation: a heritable parameter

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Abstract

Breeding programs rely on selection of individuals through their breeding values to simultaneously improve multiple traits of commercial value. In order to adequately select candidates to breed for a next generation, the genetic relationships between traits are considered in the selection index that summarizes all the traits for each selection candidate. The methods deployed in genetic evaluations rely strongly in gaussian distributions describing the data, and consider the genetic relationships between traits in the form of genetic correlations determining the joint distribution of breeding values from different traits. In this manner, genetic correlations are treated as parameters, estimated on a base population for reference. However, genetic correlations depend on the involved traits' architecture, thus depending on the genotype presented by each individual, and therefore, different individuals may present different potential for genetic correlations. Moreover, different potential for genetic correlations may partially represent a latent physiological trait responsible to balance the phenotypic expression of the measurable production traits. In practice, individual-specific genetic correlations (iSGC) can be obtained for individuals with many phenotyped descendants, as the expressed genetic correlation between the estimated breeding values among their offspring. Since the expressed iSGC depends on the involved traits' genetic architecture, part of an individual's iSGC can be transmitted to the offspring. In order to study the heritability of iSGC, two-trait genetic evaluations were performed on every pairwise combination of five traits from a French Holstein dairy cattle population: milk and protein yield (MY and PY), milking speed (MSPD), somatic cell score (SCS), and conception rate (CR). The iSGC between every pair of the five traits were obtained for ~1200 bulls with more than 500 phenotyped daughters in this population, and these iSGC were each evaluated as a phenotype with a single-trait model. This study confirmed the hypothesis that genetic correlations, when expressed as iSGC, are heritable parameters, with significant heritabilities ranging from 0.11 (iSGC between SCS and CR) to 0.51 (iSGC between PY and SCS).

Key words: Selection index, Multi-trait genetic evaluation, Genetic trade-off, Dairy cattle, Latent phenotype, Physiological traits

Introduction

Breeding programs aim to select for multiple commercial traits, in order to achieve genetic progress for all of them. Many of these traits are genetically correlated, and a negative correlation means that an antagonism between two traits exists. In dairy cattle, the genetic trade-off often lies between production and either fertility or health traits (Boichard & Manfredi, 1994; Pryce et al., 1997; Rauw et al., 1998; Roxström et al., 2001; Windig et al., 2006). Therefore, in order to avoid that selection for one trait is detrimental to the other (Hazel et al., 1994), selection must account for these negative correlations. This is typically done through a selection index, *i.e.*, a linear combination of traits, whose weights are defined by, among other information, the genetic correlations between the traits involved (Hazel, 1943; Hazel et al., 1994; Miglior et al., 2017).

Genetic correlations between traits are considered a populational parameter that defines the joint normal distribution imposed to the breeding values in genetic evaluations. However, in this manner, genetic correlations are assumed equal to all individuals, an assumption that ignores the fact that different individuals may present different physiological trade-off regulation between traits (Berry et al., 2016; Cuyabano et al., 2024). This hypothesis has been revisited by Cuyabano et al. (2024), who, in a study of the trade-off between production and fertility in the French Montbéliarde population, have shown that different sires could express different genetic correlations through their daughters, between these traits.

Because the study of Cuyabano et al. (2024) had only 247 sires with enough daughters evaluated so that reliable genetic correlations could be obtained at the individual level (*i.e.* for each sire), no further inferences could be drawn, with respect to the genetic background of these *individual-specific genetic correlations* (iSGC).

This current study hypothesized that if the different genetic correlations expressed by different sires are simply a feature of recombination and different allele frequencies in different family lines, then none or very weak heritabilities are expected to be observed for the iSGC. However, if the iSGC represent, even if only partially, a latent physiological phenotype, non-zero heritabilities should be observed for the iSGC.

To support this hypothesis that non-zero heritabilities associated to the iSGC may suggest their representation of a latent physiological phenotype, simulations were deployed. Breeding values were simulated for multiple traits, with their genetic correlations solely due to pleiotropic QTL and linkage disequilibrium between non-pleiotropic sites, in order to show that when no physiological trait was involved in the differences between genetic correlations, no heritability was captured by the iSGC.

For the real data analysis, this current study up-scaled the work from Cuyabano et al. (2024), by calculating iSGC for 1161 sires from a French Holstein dairy cattle population, between each pair of five traits of commercial interest (milk and protein yield, milking speed, cow conception rate, and somatic cell score). Heritabilities were then estimated for the iSGC, under the hypothesis that non-zero estimates suggest the representation of a latent phenotyped through the iSGC.

Materials and Methods

Bi-variate genetic evaluation model

Two-trait animal models were deployed for the genetic evaluations in this study, given by:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} g_1 \\ g_2 \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}, \qquad (1)$$

in which y_1 and y_2 are the vectors of phenotypes for traits 1 and 2 respectively; $g_1 \sim N(0, A\sigma^2_{g_1})$ and $g_2 \sim N(0, A\sigma^2_{g_2})$ are the vectors of breeding values for these two traits, with $Cov(g_1, g_2)=A\sigma_{g_{12}}$, such that A is the pedigree relationship matrix; $\sigma^2_{g_1}$ and $\sigma^2_{g_2}$ are the additive genetic variances, and $\sigma_{g_{12}}$ is the genetic covariance between the two traits; $\varepsilon_1 \sim N(0, I_n\sigma^2_{\varepsilon_1})$ and $\varepsilon_2 \sim N(0, I_n\sigma^2_{\varepsilon_2})$ are the random residuals, with $Cov(\varepsilon_1, \varepsilon_2)=I_n\sigma_{\varepsilon_{12}}$; $\sigma^2_{\varepsilon_1}$ and $\sigma^2_{\varepsilon_2}$ are the residual variances, and $\sigma_{\varepsilon_{12}}$ is the residual covariance.

The genetic evaluation model in equation (1) was implemented under a Bayesian framework, using the GIBBS3F90 module from the BLUPF90 family of (Misztal et al., 2018), with the software's default prior distributions for the breeding values and (co)variance parameters. A total of 300,000 samples were generated, with the first 100,000 discarded as burn-in. On the remaining 200,000 samples, a thinning parameter of 200 iterations was applied, resulting in 1000 effective samples used to compute the estimated breeding values (EBV) and (co)variance parameters. To assist convergence of the (co)variance parameters, initial values were provided, using the current genetic (co)variances used for these five traits in the French national genetic evaluation. Convergence was assessed visually through plots of the 1000 effective samples for the genetic (co)variances.

Heritabilities (h_1^2 and h_2^2) and genetic correlations (ρ_{12}) between the traits were obtained from the estimated (co)variance parameters, as:

$$\hat{h}_1^2=\frac{\hat{\sigma}_{g_1}^2}{\hat{\sigma}_{g_1}^2+\hat{\sigma}_{\varepsilon_1}^2} \text{ and } \hat{h}_2^2=\frac{\hat{\sigma}_{g_2}^2}{\hat{\sigma}_{g_2}^2+\hat{\sigma}_{\varepsilon_2}^2}, \qquad (2)$$

$$\hat{\rho}_{12}=\frac{\hat{\sigma}_{g_{12}}}{\hat{\sigma}_{g_1}\hat{\sigma}_{g_2}}. \qquad (3)$$

Real data

The dairy cattle data used for the present study was from the French Holstein population. The bi-variate genetic evaluations were implemented for every pair of the following five traits: milk and protein yield (MY and PY), milking speed (MSPD), somatic cell score (SCS), and cow conception rate (CR), measured as artificial insemination's success/failure on lactating cows (*i.e.* heifers excluded). The phenotypes entered for the evaluations performed in this study were in the form of yield deviations (YD), issued from the French national genetic evaluation, which evaluates MY, PY, SCS as 305-day phenotypes corrected for the duration; performance records comprise all lactations records per cow, and the model accounts for the repeatability (*i.e.*, for the permanent environment of the cow). A total of 4,501,624 cows born between 1991-2020 had YD deviations available for all five traits, with a pedigree file containing a total of 8,275,018 animals that traced back three generations from the cows with performances.

Simulated data

The simulated data consisted of ten replicates of populations with a founder population followed by 30 generations under selection. Generations were non-overlapping, each with 1000 individuals, among them 200 males and 800 females. Selection was performed at each generation for the top 20% males, based on a selection index build from their true simulated breeding values, assuming equal weights for all simulated traits. Pedigree information was kept for the simulated populations.

Five traits were simulated with additive effects associated to them, and genetic correlations were solely due to pleiotropic QTL and linkage disequilibrium (LD) between non-pleiotropic sites. To simulate these traits, 1675 SNP genotypes, already in LD from the founder population (average LD of 0.15 in this population), were simulated to serve as quantitative trait loci (QTL). At each population replicate, a random subset of 75 SNPs were assigned as pleiotropic QTL across all five traits, five random subsets of 25 SNPs each were assigned as pleiotropic QTL across four traits, ten random subsets of 50 SNPs each were assigned as pleiotropic QTL across three traits, and ten random subsets of 90 SNPs each were assigned as pleiotropic QTL across two traits. The remaining 75 SNPs were finally split in five groups of 15, to be assigned as QTL exclusive to each one of the five traits. This distribution of the QTL per trait is presented in the Venn diagram in Figure 1.

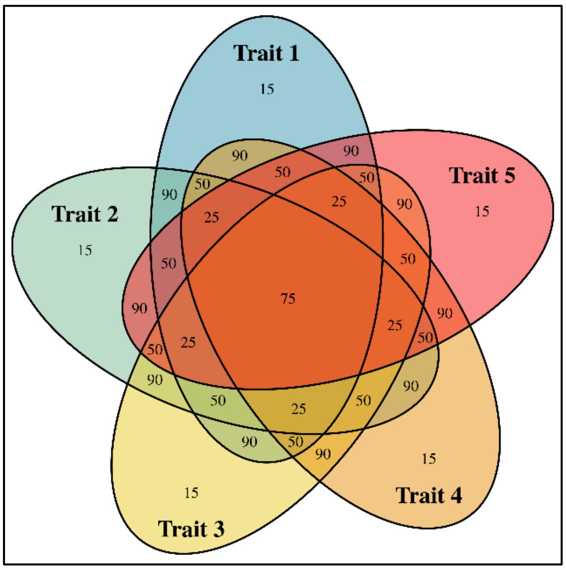

Figure 1. Venn Diagram describing the number of QTL shared among the five simulated traits.

Finally, QTL-effects were simulated, correlated between traits, so that the breeding values at the founder population presented genetic correlations matching those obtained for the traits studied in the French Holstein dairy cattle population. The additive genetic variances of the simulated breeding values were set to $10 \times h^2$, with the heritabilities being those obtained for the real traits evaluated from the French Holstein dairy cattle data.

Individual-specific genetic correlations and their heritability estimates

For both the real and simulated data, *individual-specific genetic correlations* (iSGC) were calculated for sires, in order to evaluate how much differences in genetic correlations were expressed by different sires.

For the real data, iSGCs were calculated for all pairs of the five traits, evaluated with the bi-variate genetic evaluations models given by equation (1). Following the proposed by Cuyabano et al. (2024), sires with more than 500 daughters evaluated were selected, so that reliable genetic correlations could be obtained at the individual level, based on the daughters' EBVs. A minor change was made to calculate the iSGC, compared to how it was done by Cuyabano et al. (2024), who obtained the iSGC per sire by correlating the EBVs from their daughters. Here, prior to calculating the correlations between the daughters' EBVs from different traits, half of the dam's EBVs were subtracted from their daughters, so that on average, the iSGC comprised only sire information. Thus, for each sire s and for any pair of traits 1 and 2, their $i\text{-}th$ daughter's breeding values were corrected as:

$$\hat{g}_{1i,s}=\hat{g}_{1i}-\frac{\hat{g}_{1\{dam\ of\ i\}}}{2}, \quad (4)$$

$$\hat{g}_{2i,s}=\hat{g}_{2i}-\frac{\hat{g}_{2\{dam\ of\ i\}}}{2}, \quad (5)$$

for every i=1, ..., n_s. Finally, for each sire s:

$$iSGC_s=\frac{\sum_{i=1}^{n_s}\left(\hat{g}_{1i,s}-\overline{g}_{1,s}\right)\left(\hat{g}_{2i,s}-\overline{g}_{2,s}\right)}{(n_s-1)\hat{\sigma}_{g_{s1}}\hat{\sigma}_{g_{s2}}}, \quad (6)$$

such that $\overline{g}_{1,s}=\frac{\sum_{i=1}^{n_s}\hat{g}_{1i,s}}{n_s}$ and $\overline{g}_{2,s}=\frac{\sum_{i=1}^{n_s}\hat{g}_{2i,s}}{n_s}$ are the mean daughters' corrected EBVs, and their variance are $\hat{\sigma}^2_{g_{s1}}=\frac{\sum_{i=1}^{n_s}\left(\hat{g}_{1i,s}-\overline{g}_{1,s}\right)^2}{n_s-1}$ and $\hat{\sigma}^2_{g_{s2}}=\frac{\sum_{i=1}^{n_s}\left(\hat{g}_{2i,s}-\overline{g}_{2,s}\right)^2}{n_s-1}$.

For the simulated data, iSGCs were calculated for all pairs of the five traits, only for the selected sires in the simulation routine. Since the simulations provided genotypes and the true simulated QTL effects, instead of using daughters' information, for each sire s, 500 gametes were simulated, at which QTL effects were applied. Thus, for each sire s and for any pair of traits 1 and 2, the additive genetic values of the $i\text{-}th$ gamete was given by:

$$\gamma_{1i,s}=\sum_{j=1}^{1675} X_i\alpha_{1j}, \quad (7)$$

$$\gamma_{2i,s}=\sum_{j=1}^{1675} X_i\alpha_{2j}, \quad (8)$$

for every i=1, ...,500, such that α_{1j}'s and α_{2j}'s are the QTL effects (set as zero if the $j\text{-}th$ SNP is not a QTL for each of the traits). Finally, for each simulated sire s:

$$iSGC_{s(\sim)}=\frac{\sum_{i=1}^{500}\left(\gamma_{1i,s}-\overline{\gamma}_{1,s}\right)\left(\gamma_{2i,s}-\overline{\gamma}_{2,s}\right)}{(499)\hat{\sigma}_{\gamma_{s1}}\hat{\sigma}_{\gamma_{s2}}}, \quad (9)$$

such that $\overline{\gamma}_{1,s}=\frac{\sum_{i=1}^{500}\gamma_{1i,s}}{500}$ and $\overline{\gamma}_{2,s}=\frac{\sum_{i=1}^{500}\gamma_{2i,s}}{500}$ are the mean additive genetic values of the gametes, and their variance are $\hat{\sigma}^2_{g_{s1}}=\frac{\sum_{i=1}^{500}\left(\gamma_{1i,s}-\overline{\gamma}_{1,s}\right)^2}{499}$ and $\hat{\sigma}^2_{g_{s2}}=\frac{\sum_{i=1}^{500}\left(\gamma_{2i,s}-\overline{\gamma}_{2,s}\right)^2}{499}$.

Heritability estimates were obtained for the iSGCs, by treating them as a phenotype in a variance component estimation routine, using the pedigree relationship matrix for both the real and the simulated data, tracing back four generations from the sires. For the simulated data, heritability estimates were also obtained using a genomic relationship matrix (VanRaden, 2008) built from the simulated SNP-genotypes. The following model was used to estimate variance components:

$$iSGC=1_n\mu+g+e, \quad (10)$$

in which $iSGC$ is the vector of iSGCs obtained for the n sires, between any two traits; μ is the overall mean; $g \sim N(0, A\sigma^2_{g(iSGC)})$ is the vector of breeding values associated to the iSGC, A is the pedigree relationship matrix (replaced by the genomic relationship matrix G, for the simulated data), and $\sigma^2_{g(iSGC)}$ is the additive genetic variance associated to the iSGC; and $e \sim N(0, I_n \sigma^2_{e(iSGC)})$ is the vector of random residuals, and $\sigma^2_{e(iSGC)}$ is the residual variance.

Variance components for the iSGC were estimated through the residual maximum likelihood (REML; Patterson & Thompson, 1971), using the REMLF90 module from the BLUPF90 family of programs (Misztal et al., 2018). Finally, heritabilities of the iSGC were given by:

$$\hat{h}^2 = \frac{\hat{\sigma}^2_{g(iSGC)}}{\hat{\sigma}^2_{g(iSGC)} + \hat{\sigma}^2_{e(iSGC)}}. \qquad (11)$$

Results & Discussion

Genetic parameters on real data

Heritability and genetic correlation estimates were obtained from the genetic parameters of the bi-variate genetic evaluations, for every pair of the five traits studied from the French Holstein dairy cattle population, and their values are presented in Table 1. These values agreed with those used for the French national genetic evaluation, as expected, and also agreed with reported heritabilities and genetic correlations between these traits. Finally, these values presented in Table 1 were the ones used as parameters to generate the breeding values for the simulated data, with genetic variances equal to $10 \times h^2$.

Table 1: Estimated heritabilities (diagonal bold values) and genetic correlations (upper triangle of the table) between the five traits studied in the French Holstein dairy cattle population. Values in gray indicate an estimate that was not statistically different from zero (significance level of 0.05).

	MY	PY	MSPD	SCS	CR
MY	**0.22**	0.78	-0.06	-0.04	-0.15
PY	---	**0.38**	-0.07	-0.01	-0.20
MSPD	---	---	**0.24**	0.31	-0.04
SCS	---	---	---	**0.13**	-0.26
CR	---	---	---	---	**0.01**

Distribution of the individual-specific genetic correlations on real and simulated data

Figures 2-5 present the distributions, in the form of density curves, of the iSGC obtained between the five traits studied, both on real and simulated data, indicating that different sires did present different potential for genetic correlations, expressed through their offspring.

The mean iSGCs on the real data presented bigger differences from the estimated genetic correlations with the Gibbs sampler, presented in Table 1 and indicated with dots at the x-axes of the plots, than the mean iSGCs on the simulated data. This could be due to the fact that, on real data, iSGCs were obtained for a subset of sires that had at least 500 daughters evaluated, rather than for all sires, potentially indicating a different mean iSGC for these elite sires, with respect to the whole population.

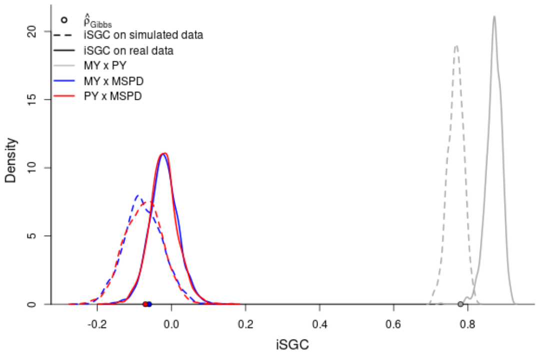

Figure 2. Distribution of the iSGC obtained across the pairs of the three production traits (MY, PY, and MSPD), on both real and simulated data.

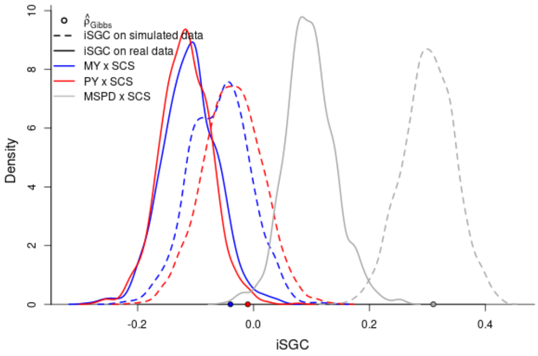

Figure 3. Distribution of the iSGC obtained between the production traits (MY, PY, and MSPD) and the health trait (SCS), on both real and simulated data.

Interestingly, on the real data, the overall iSGC between MY and CR and between PY and CR were less negative for the elite sires than the estimated genetic correlations between these traits, as shown in Figure 4. Conversely, the overall iSGC between MY and SCS and between PY and SCS were rather more negative (*i.e.* a stronger trade-off between these traits) for these elite sires than the estimated genetic correlations between these traits, as shown in Figure 3. If the hypothesis that iSGCs express a latent physiological trait holds, even if at least partially, these results suggest that selection is favoring a physiological trait that allows a better management of the trade-off between production and fertility, however in the detriment of the trade-off between production and health indicators. Nonetheless, it is important to note that a strengthening of the trade-off between traits does not mean that the traits themselves are not achieving genetic progress.

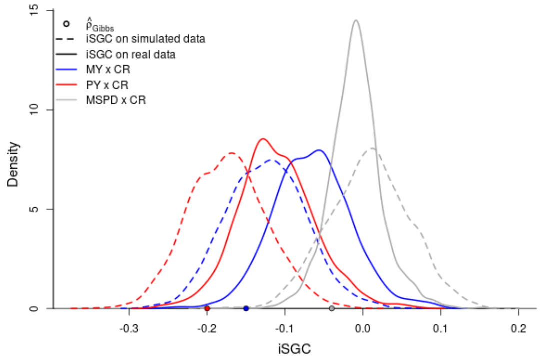

Figure 4. Distribution of the iSGC obtained between the production traits (MY, PY, and MSPD) and the fertility trait (CR), on both real and simulated data.

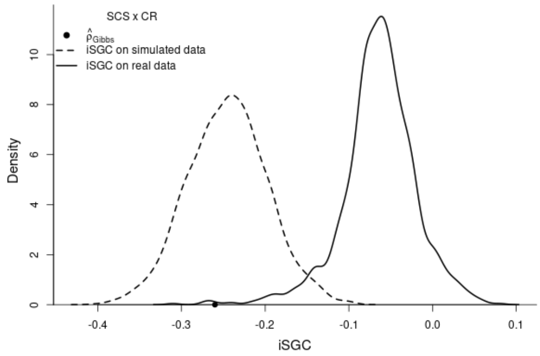

Figure 5. Distribution of the iSGC obtained between the health and fertility traits (SCS and CR), on both real and simulated data.

Heritabilities of the individual-specific genetic correlations on real and simulated data

Heritabilities were estimated for the iSGC, by treating them as a phenotype, as in the model presented in equation (10). These heritabilities were estimated for the iSGC obtained for both the real and simulated data. The goal of comparing these heritabilities of the iSGC on real data, to those of the iSGC on simulated data with the same genetic parameters, was to show that when no latent trait was associated to the differences between genetic correlations in a population, no heritabilities would be captured.

The estimated heritabilities are presented in Table 2, being the presented values for the heritabilities of iSGCs obtained on simulated data (lower triangle of Table 2), the obtained using the pedigree relationship matrix, since their values were not statistically different from the obtained with the genomic relationship matrix (significance level of 0.05). All these heritabilities of the iSGC on simulated data were not statistically different from zero (significance level of 0.05), indicating that neither family relationships, nor allele frequencies and LD patterns were enough to outline a genetic determinism for the different iSGC expressed by different sires.

With respect to the heritabilities of the iSGC on real data (upper triangle of Table 2), their values were significantly different from zero (significance level of 0.05), with the exception of the heritability of iSGC between MSPD and CR. Particularly, heritabilities of the iSGC between the two main production traits (MY and PY), between these main production traits and the health trait (SCS), and between these main production traits and the fertility trait (CR), were moderately high for dairy cattle traits, ranging from 0.38 to 0.51. These heritabilities suggest a reasonable level of genetic determinism associated to the different iSGC expressed by different sires, and these heritabilities could be due to the genetic correlations at the individual level expressing, at least partially, a latent physiological trait.

Table 2: Heritability estimates for the iSGC obtained on the real data (upper triangle of the table), and for the iSGC obtained on the simulated data (lower triangle of the table). Values in gray indicate an estimate that was not statistically different from zero (significance level of 0.05).

	MY	PY	MSPD	SCS	CR
MY	---	0.45	0.16	0.45	0.46
PY	0.03	---	0.17	0.51	0.38
MSPD	0.02	0.02	---	0.23	0.05
SCS	0.02	0.02	0.02	---	0.11
CR	0.02	0.03	0.03	0.02	---

Conclusions

Genetic correlations, while treated as a parameter common to all individuals in genetic evaluations and selection indexes, may present different values across individuals in a population. By obtaining individual-specific genetic correlations for sires from a French Holstein dairy cattle population, this study has shown that indeed, different individuals present different patterns in their genetic correlations between five traits of interest. Moreover, individual-specific genetic correlations are heritable, suggesting that these parameters may be part of the expressions of a non-measurable (or latent) physiological trait. When it comes to traits that present a negative genetic correlation, the findings from this study may assist to select individuals better apt to manage the trade-off between traits. However, it remains a question of research, how to adequately and optimally use individual-specific genetic correlations and their heritability in a breeding program.

Acknowledgments

This project has received funding from the European Union’s Horizon 2020 Programme for Research & Innovation under grant agreement n°101000226.

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Implementation of single-step genetic evaluation in Poland

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Abstract

In April 2025, Centre for Genetics of Polish Federation of Cattle Breeders and Dairy Farmers (CGen) implemented single-step genomic evaluation for Polish Holstein-Friesian population. The process of implementing the new evaluation system started in December 2024 when CGen became the official provider of dairy genetic evaluations in Poland calculating selection indexes based on breeding values estimated by the National Institute of Animal Production in Balice using a two-step approach. To increase the accuracy of breeding value estimations, CGen developed single-step pipelines using BLUPF90 family of programs. In January 2025 conventional EBVs for all traits were submitted for Interbull test run and successfully passed the evaluation. Next, the results of genomic evaluations were submitted for GEBV test and also passed for all traits. In April 2025 single-step evaluation results were officially published for the industry. In this paper we share the experience from the work undertaken to implement the single-step approach in Poland. The changes to the genetic evaluation models are highlighted. The methodology implemented for the integration of external information (MACE proofs) in single-step evaluations is discussed and results presented. Technical aspects of implementation are also discussed including model running times for individual traits. Highlights from the validation work undertaken are also included. The most noticeable impact on genetic evaluation results of the transition from the two-step evaluation system to the single-step method was increase in reliabilities. For example, for production traits, reliability increased by 12 percentage points for bulls and 10 percentage points for cows. The most significant improvement was observed for longevity, with a reliability increase of 40 percentage points for bulls and 35 percentage points for cows. The new system also demonstrated substantial improvement in the correlation between Polish and international breeding values, as estimated by the Interbull Centre. The most noticeable improvement was for longevity, where correlation increases with some countries reached 46 percentage points.

Key words: genetic evaluation, genomic parameters, single-step evaluation, dairy

Introduction

The implementation of the single-step genetic evaluation in Poland was a significant project undertaken by several organizations, led by the Centre for Genetics of the Polish Federation of Cattle Breeders and Dairy Farmers (CGen) and supported by the National Research Institute of Animal Production in Balice, Poznan University of Life Sciences, Krakow Agricultural University, Wroclaw University of Life Sciences, and AbacusBio Ltd. This initiative aimed to transition from a two-step to a single-step genomic evaluation system for Holstein-Friesian cattle, using the BLUPF90 family of programs (Aguilar *et al.*, 2018). The take-over of the routine genetic evaluation by CGen followed the European trend in which breeders' associations are taking responsibility for the national evaluations. Similar changes were implemented in France, Netherlands, or Germany. The final change of the evaluation unit was accepted by the Ministry of Agriculture in December 2024, and the implementation of the new system took place in April 2025 after a successful validation at the Interbull Centre.

The transition to a single-stage system included: implementing BLUPF90 family of programs, change in definition of fertility traits and longevity, changes in models used to evaluate some traits e.g. production and conformation, but also introducing new traits (digital dermatitis (DD), bone quality, and interval from 1st to last insemination. One of the main issues was integration of external breeding values i.e. MACE proofs as bulls' pseudo-phenotypes. In this paper we show the results of implementing the single-step genomic evaluation based on a few selected from all the 47 traits included in the Polish national genetic evaluation system.

Materials and Methods

The population of Polish Holstein-Frisian cows are under routine recording, performed by Polish Federation of Cattle Breeders and Dairy Farmers (PFHBiPM). The database contained over 4M cows with phenotypes. The number of records varied between traits, for example the data set for production traits had over 76M records and for DD 270K. The 3-5 generations of pedigree, depending on the trait, included in the analyses was created based on own herdbook data and integrated with Interbull and EuroGenomics pedigree data including 6M animals. More than 240K genotypes were used in the analyses which comprised of genotypes of Polish cows and bulls supplemented by EuroGenomics' data and data from other international exchanges.

Models

We employed a range of animal models depending on the analyzed trait. In the case of production traits, we employed multi-lactation random regression test-day models with lactation curve modelled with Legendre Polynomials. Calving traits were multi-lactation models with maternal effect. Type traits were analyzed using multi-trait models with correlated traits connected in blocks. For fertility traits, longevity and digital dermatitis we used single trait multi-lactation models. Workability traits were analyzed with single-trait animal models.

Software

The variance components and breeding values were estimated using BLUPF90 family of programs. The variance components were estimated using Gibbs sampling algorithm with GIBBSF90+, BLUP90IOD3 to solve mixed models and ACCF90GS3 to approximate reliability including genomic information. The genomic runs utilized APY (Algorithm for Proven and Young) algorithm for genomic evaluations (Misztal *et. al.*, 2014, Misztal, 2016). The core for the APY consisted of 18K animals, which is related to the number of independent chromosome segments (haplotypes) in bovine, this number of core animals allowed to capture 99% of genetic variation (Pocrnic *et al.*, 2016). The list of the core animals was created using a strategy that prioritized animals from the reference population common across seven different trait groups. A "reference list" was first built by randomly sampling animals present in the most trait groups, with special consideration for key traits like digital dermatitis, conformation and fertility. This list was then supplemented with a selection of the most popular insemination bulls to finalize the sample.

MACE Integration

MACE breeding values obtained from the Interbull Centre were used as bulls' pseudo-phenotypes in order to integrate international breeding values in the national genomic evaluation following Bonifazi *et al.* (2023). In short, the international and national breeding values for bulls were de-regressed using their reliability. Then the national de-regressed proofs were used to remove the contribution of Polish cows to the MACE proofs. The MACE proofs were used only if they were more accurate than the national proofs. The converted MACE proofs were weighted using their reliability and included in the data as bulls' phenotypes. All bulls were assigned to one contemporary group to account for differences in the average level between phenotypes and the de-regressed proofs. In the case of random-regression models, we additionally converted the de-regressed proofs such that they were on the level of the test-day records instead of the full lactation EBV using Eding (2024) approach.

EBV Standardization

The final breeding values were standardized using the rolling base population that consists of cows with phenotypes born 10 years before the evaluation and will be updated on a yearly basis. All traits except milk production traits are expressed on a scale with the mean of 100 and SD of 10.

Results and Discussion

Selection of traits for comparison

To show the outcomes of implementing single-step evaluation system in Poland we present results for a few selected traits only. We selected: milk yield, longevity, and digital dermatitis (DD). Milk yield was included due to its extensive history in Polish genetic evaluation, having undergone moderate modifications (comparing to changes in other trait groups) regarding data filtering and model in new system. This provides a stable baseline for comparison. The heritability for milk yield was estimated at the level of 0.37. Longevity was chosen as a trait with a substantial period of being included in the evaluation in Poland and a comprehensive phenotypic data set. Notably, the current evaluation for longevity has undergone a complete revision, including changes in trait definition, data filtering and variance components estimation (Stachowicz *et al.*, 2024). The heritability level for longevity was estimated to be 0.16. The third trait, digital dermatitis, represents a recent addition to the genetic evaluation with breeding value evaluation started in 2024. Phenotypic data for DD have been incorporated into the evaluation system since 2018. It represents low heritable traits with a heritability of 0.07.

EBV correlations with previous system

The introduction of the new single-step system caused significant re-ranking of animals. Correlation between indexes from previous and new system ranged from 0.83 to 0.96 depending on focal group of animals. In the top 200 ranking bulls only 96 were in common between the two systems and only 66 females. Correlations for production traits were the highest, ranging from 0.88 for protein to 0.94 for fat. The larger changes were observed for longevity and fertility with correlations of 0.6 and 0.5, respectively.

MACE Integration results

Correlation between domestic pedigree-based (conventional) evaluations and international proofs for bulls with daughters in Poland for milk production was 0.99. The same correlation for international bulls without daughters in Poland was 0.79. The inclusion of MACE proofs in single-step evaluation resulted in an increase in the correlation with MACE proofs to 0.98. For longevity the corresponding value increased from 0.72 to 0.96, respectively.

Genetic correlations with other countries

For production traits the correlations between countries from previous and current system are high and stable, at approximately 0.9 for milk yield (ranged from 0.87-0.9). The lowest correlation was obtained between Poland and New Zealand 0.66 for both current and previous systems. This means that the changes introduced did not significantly impact the results obtained in Poland and are consistent with the evaluation obtained in other countries. For traits where significant changes were made in the definition, model, or data editing, such as longevity, the improvement in correlation is significant, ranging from approximately 0.2 to 0.45. The highest increase was observed between Poland and counties: USA, Germany, DFS and Spain. This means that the changes introduced positively influenced the estimated breeding value results.

Digital dermatitis is a new trait evaluated in Poland since April 2024. Based on results from the Interbull research run for new traits Poland received very high correlations for digital dermatitis with six other countries participating in the study, with the highest correlation being 0.93 with the Netherlands. These results confirm that the breeding value estimated in Poland is consistent with those estimated in other countries.

Reliability

A notable observation in the current genomic evaluation system is an increase in reliability, particularly for genotyped animals and reference population. This improvement is evident in both male and female. For milk yield, the average increase in reliability was 10 and 12 percentage points for bulls and cows, respectively, when comparing the previous two-step evaluation system to the current single-step approach. In the case of longevity, this increase in reliability is substantial, averaging 40 percentage points for bulls and approximately 35 percentage points for females. As mentioned, digital dermatitis has not previously undergone a two-step evaluation, which makes it impossible to directly compare the reliability of both systems. However, Figure 1 illustrates the distribution of genomic breeding value reliability for females derived from the single-step system. As shown, females with only phenotypes showed the lowest reliability, with an average reliability of 0.4 in this group. In contrast, females from the reference population (having both the phenotype and genotype) show the highest reliability, averaging around 0.7. Females with only a genotype achieve an average reliability of approx. 0.6. These reliability levels are notable given that DD is a low-heritability trait with a relatively small number of recorded phenotypes. For all 47 traits currently under evaluation, an observed increase in reliability ranged from 5 to 40 percentage points.

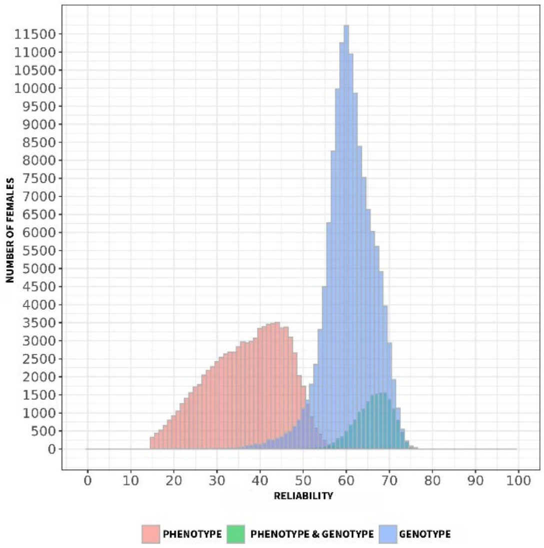

Figure 1. Reliability of the genomic breeding values for digital dermatitis in three groups of Holstein-Friesian females.

Run times

The implementation of the APY (Algorithm for Proven and Young) approach, combined with multi-core processing options within the BLUP90IOD3 software, has resulted in relatively short computational times for full genomic evaluation runs. The processing time varies depending on the trait under consideration, ranging from (in clock hours): 1.5h for longevity, to 5h for digital dermatitis (DD), and between 13h to 33h for production traits.

Conclusions

Implementation of single-step evaluation system utilizing APY algorithm allowed to improve the national genetic evaluation system in Poland. The international correlations estimated by Interbull Centre for conventional proofs, generally, increased. The genomic breeding values reliability increased substantially thanks to including phenotyped and genotyped cows in the reference population while utilizing MACE proofs for bulls evaluated based on foreign daughters. Including cows in the reference population allowed for a successful implementation of hoof health trait which is not a routinely recorded trait in Poland.

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Implementation of a Single-Step genomic evaluation system for dairy cattle in Wallonia, Belgium

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Abstract

For several years, dairy cattle breeders in the Walloon Region of Belgium have had access to locally estimated breeding values (EBV) for traits of interest. These evaluations enable Wallonia to contribute to the Multiple Across Countries Evaluation (MACE) conducted by Interbull. In the current local genomic evaluation framework, genomic and pedigree data are integrated with local EBV and external information, MACE-derived EBV (MACE EBV), through a pseudo-single-step genomic evaluation system, producing genomically enhanced EBV (GEBV). However, this approach may introduce biases. To address this, the present study aimed to implement a single-step genomic BLUP (ssGBLUP) that simultaneously incorporates all available national data alongside MACE information and validate this method using milk production traits. The proposed strategy first involves defining "pseudo-traits" that represent MACE traits (i.e., 305-day averages for milk, fat, and protein yields over the first three lactations). MACE EBV are then transformed into adjusted pseudo-phenotypes (i.e., deregressed proofs) and effective contributions but avoiding double-counting of Walloon data within MACE EBV. Next, the variance-covariance matrices from the local random regression test-day model were modified to include the three MACE pseudo-traits as correlated traits. Finally, a single-step genomic evaluation was performed, jointly analyzing test-day records and MACE pseudo-phenotypes. Validation of both pedigree-based and single-step genomic evaluations, both integrating MACE information, was carried out using data from the official Walloon genetic evaluations of April 2022. Results show that MACE information is adequately integrated in the local evaluations, because Pearson correlations between MACE EBV and the integrated EBV were higher than 0.97 across traits. The addition of genomic information in single-step evaluations resulted in small changes for all individuals, as illustrated by Pearson correlations ranging from 0.975 and 0.986 for sires with MACE information.

Key words: dairy cattle, single-step GBLUP, multiple across-country evaluation, milk production traits

Introduction

Walloon Holstein dairy cattle breeders are provided with locally estimated breeding values (EBV) for production-, conformation-, udder health and other functional traits. Nearly all traits are submitted to Interbull (https://interbull.org, Uppsala, Sweden), which performs an across-country genetic evaluation using a multiple across-country evaluation (MACE) system, resulting in MACE-derived international EBV hereafter called MACE EBV (Schaeffer, 1994).

In the current Walloon genomic evaluation system, these MACE EBV are combined with pedigree and genomic information in a pseudo single-step evaluation using a Bayesian integration procedure, accounting for double-counting, to produce local genomically enhanced EBV (GEBV) (Vandenplas et al., 2014; Colinet et al., 2018). This system enables the integration of external (international) information across all animals and datasets in the local system, ultimately enhancing selection decisions (Vandenplas et al., 2015; Vandenplas et al., 2017; Bonifazi et al., 2023). Moreover, post-processing steps are performed to integrate MACE, GMACE and local EBV/GEBV before publication.

However, this pseudo single-step evaluation may introduce biases. In particular, this approach still relies on a first BLUP step which does not estimate fixed effects accounting correctly for genetic differences as reflected by final GEBV. To address this, single-step GBLUP (ssGBLUP) is the method of choice as it combines phenotypic data, pedigree and genomic information simultaneously, resulting in GEBV that are both more accurate and less biased (Misztal et al. 2009, Aguilar et al, 2010, Christensen and Lund, 2010).

The objectives of this study were to develop and validate a single-step genomic evaluation that simultaneously incorporates all available local data alongside MACE information for milk production traits in Walloon Holstein dairy cattle. Integration of MACE information in the current system is very important and had to be conserved. This study is part of an ongoing effort to implement this strategy for all our current evaluations but also for novel traits such as enteric methane emissions.

Materials and Methods

Data

Phenotypic data

All data were provided by the Walloon breeding association Elevéo (Awé Groupe, Ciney, Belgium), which manages performance recording data in the Walloon region of Belgium. Data used in the study were from the official Walloon routine April 2022 genetic evaluations.

Three types of data were used: 1) 4 851 501 test-day records for 305-day milk (MY), fat (FY), and protein yields (PY) across three lactations, 2) MACE EBV and reliabilities of 12 547 bulls obtained from Interbull for these traits, and 3) local EBV and reliabilities of 2 230 local Walloon bulls that were sent to Interbull.

Furthermore, the cleaned pedigree used for the April 2022 routine evaluation consisted of 4851501 animals. Genetic groups were defined as in the current evaluations by group of birth years, origin (Europe vs. USA) and sex.

Genomic data

Genomic data were available for 13 604 animals and consisted of 30 554 single nucleotide polymorphisms (SNPs), routinely used in the Walloon genetic evaluation system. Genotyping was done using the BovineSNP50 Beadchip v1 to v3 and EuroG MD (SI) v9 (Illumina) chips. SNPs common across the four chips were retained, while non-mapped SNPs, SNPs located on sex chromosomes, and triallelic SNPs were excluded. SNPs exhibiting Mendelian conflicts or with a minor allele frequency less than 5% were excluded. The difference between observed and expected heterozygosity was estimated, and SNPs with a difference greater than 0.15 were excluded (Wiggans et al., 2009). After applying all quality control measures, non-mapped SNP, SNP located on sex chromosomes, SNP with Mendelian conflicts, and those with minor allele frequency less than 5% were excluded. Finally, data of 28 470 SNPs located on 29 chromosomes were used.

Definition of MACE Pseudo-traits

Adding external information from MACE discounting for already contained local information has been a topic of applied research for a long time (Gengler & Vanderick, 2008). Recently, a commonly accepted strategy was developed that we also implemented. Following the framework of Vandenplas et al. (2015), MACE EBV were transformed into deregressed proofs (DRP), i.e., “pseudo-phenotypes”, for MY, FY, PY for MACE bulls excluding local information. These pseudo-traits captured the genetic signal embedded in MACE EBV, while avoiding double-counting of national records. They were associated to weights called effective record contributions (ERC), expressing how many record equivalences would have generated the same information content.

Following the strategy developed by Bonifazi et al. (2023), deregression was performed using the method of Garrick et al. (2009) where EBV were adjusted by their reliability to produce DRP. There are two inherent issues that Bonifazi et al. (2023) solved: the elimination of local information in MACE EBV, and the deregression applied to EBV corrected for parent averages (PA), i.e. Mendelian Sampling deviation. Reliability (REL) of this deviation is needed in the deregession process. This value can be obtained by transforming REL to ERC and subtracting ERC associated with PA REL from total ERC. This yields dERC, necessary to properly weigh the DRP used as pseudo-phenotypes in an evaluation. In a final step double counting is eliminated by subtracting local information sent to Interbull generating DRP* and dERC* free of local information.

Models and analysis

The model currently used in the Walloon region of Belgium is a three-lactation, three-trait random regression test-day model (RRTDM) for milk, fat and protein yields. Average lactation EBV are generated computing weighted sums of the underlying genetic random regression solutions resulting in aggregate 305-DIM three-lactation EBV that are sent to Interbull (EBV_sent). Details can be found in Auvray & Gengler (2002) and Croquet et al. (2006).

We developed the new model to be as close as possible to the current Walloon genomic evaluation, which uses a pseudo-single-step evaluation as outlined in Vandenplas et al. (2014) and Colinet et al. (2018). Briefly explained, local EBV and MACE EBV were combined in a system using a combined pedigree and genomic relationships matrix, while discounting for local information included in MACE (EBV_sent), resulting in local GEBV. An overview of this system can be found in Figure 1A.

The new developed system relies on the simultaneous analysis of phenotypic data, instead of local EBV, the MACE pseudo-phenotypes (as DRP) weighted by ERC, and pedigree and genomic information using ssGBLUP (Aguilar et al., 2010), resulting in GEBV (GEBV_ss).

Another problem is that the local traits and MACE traits are not on the same scale. Recently some research (e.g. Boerner et al., 2023) has proposed complex solutions. The complication is, however, only because one tries to pass from a 305DIM 3-lactation MACE trait represented by a single EBV to a large number of genetic effects (i.e., 3 random regressions) and traits (i.e. 3 lactations). Complicated and potentially imprecise backsolving equations can be avoided by using the following process. The variance-covariance matrices from the national test-day random regression model were modified to include the three MACE pseudo-traits as correlated traits but initially without records linking these to the Legendre polynomials across the 3 lactations. As the linear function in the direct local evaluation solutions to MACE EBV is a simple transformation, transforming by the same function initial variance-covariance matrices generates the needed augmented genetic (co)variance matrices (Vandenplas et al., 2015). Its singularity was avoided by multiplying the used (co)variance between random regressions and MACE traits by a factor of 0.999. An interesting side effect of this reparameterization is that the new system of equation generates natively EBV to be sent to the MACE runs of Interbull. Moreover, the setting of this equation system is very flexible, so, one can choose to add or not MACE bulls. The system of equations can also be easily modified to keep fixed effects constant. This will allow us to provide Interbull with the different requested unbiased EBV in the future (i.e., computing EBV_sent but using fixed effects that are obtained by solving with **H**).

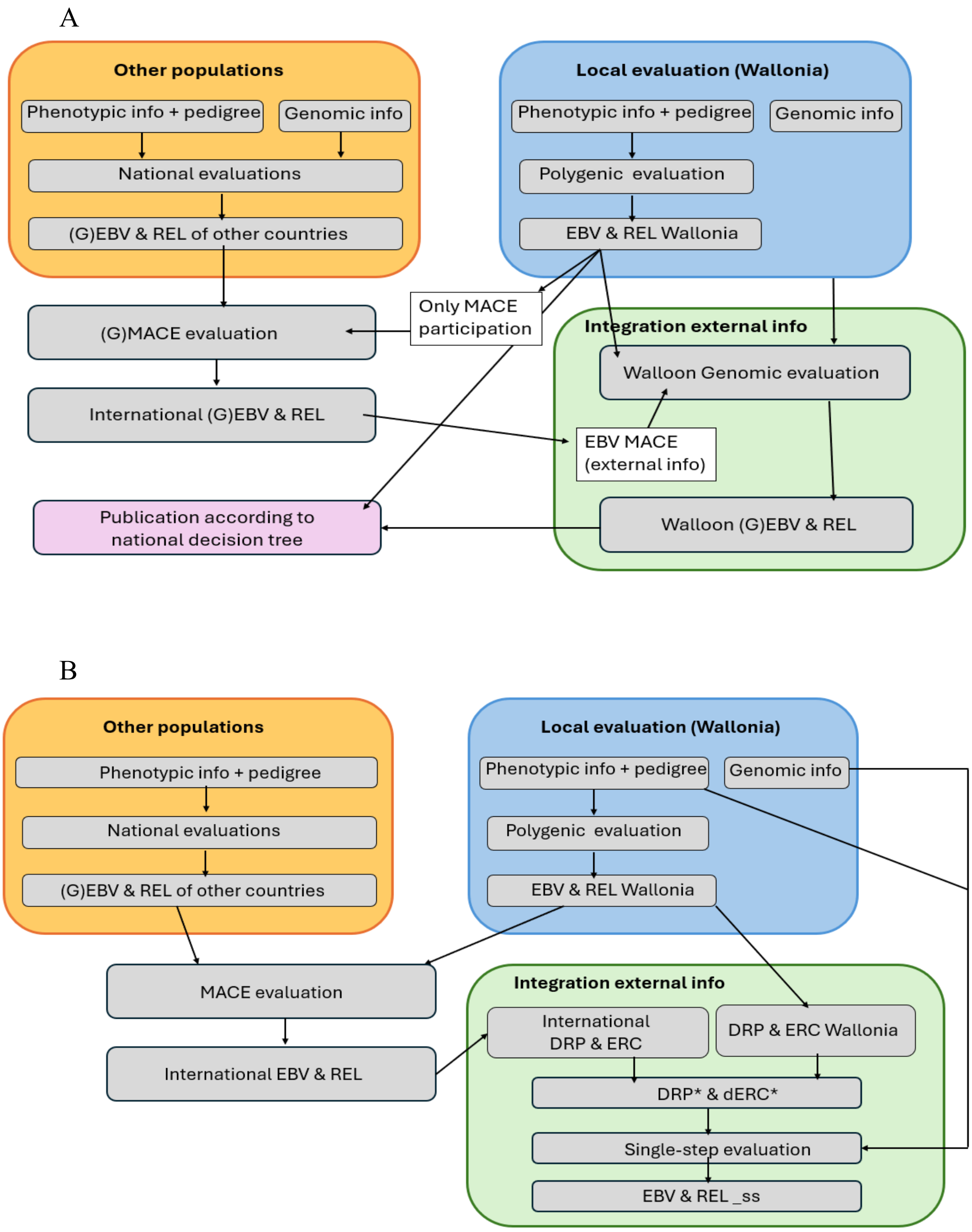

Figure 1. Comparison of the currently applied genomic evaluation system in Wallonia (above, A), compared to the developed single-step system with integration of MACE information based on DRP (below, B).

Figure 1B gives an overview of the developed single-step evaluation system with incorporation of MACE data based on DRP* and dERC*. Software used for these calculations was based on BLUPF90 family of programs (Misztal et al., 2014) but with a certain number of adaptations including checking of residuals for outliers and other adaptations to improve its usefulness in the context of genetic evaluations.

Furthermore, the calculation of genomic reliabilities had to be adapted as well. First, pedigree reliability (REL) has been determined using Effective Daughter Contributions and following standard procedures employed in the current genetic evaluations for yield traits in the Walloon Region of Belgium, as described by Strandén et al. (2000), but extended to allow integration of ERC of MACE bulls. Second, genomic reliability (GREL) was calculated using an approach adapted from Gao et al. (2023), using the previously obtained pedigree REL as a starting value after adjustment for double-counting due to pedigree information (Zaabza et al., 2022).

Validation

To assess consistency, we did two types of comparisons. First, to validate the integration of MACE information in a genetic evaluation, we compared for the same bulls MACE EBV with EBV_ss obtained from a pedigree-based evaluation integrating MACE information as in the developed ssGBLUP. Theoretically, the system should yield exactly the same EBV_ss to MACE EBV. Then, the GEBV from the current genomic evaluation system were compared to GEBV_ss obtained using the developed ssGBLUP system (GEBV_ss), both systems integrating EBV MACE. In this study we compared correlations and regression coefficients, the first establishing the relationship between sets of breeding values, the later assessing potential changes in variances (i.e., inflation of deflation) of the sets of breeding values that are compared.

Results

Validation of integration of MACE information in the genetic evaluation

Tables 1 and 2 present Pearson correlations among EBV obtained from the current local pedigree-based evaluation (EBV_sent), MACE EBV, EBV obtained from the pedigree-based evaluation integrating MACE EBV (EBV_ss), and GEBV obtained from the new ssGBLUP with integration of MACE EBV (GEBV_ss). Table 1 specifically shows the correlations for milk, fat and protein yields for all bulls included in the MACE evaluation, as well as for own local bulls participating in MACE.

Table 1: Pearson correlations between (G)EBV_ss with MACE EBV and EBV_sent, for MACE bulls, with or without any local information sent to INTERBULL, for milk (MY), fat (FY) and protein (PY) yields.

		MACE EBV (N=12547)	EBV_SENT (N=2230)
MY	EBV_ss	0.967	0.962
	GEBV_ss	0.969	0.960
FY	EBV_ss	0.945	0.958
	GEBV_ss	0.950	0.955
PY	EBV_ss	0.979	0.964
	GEBV_ss	0.980	0.961

Table 2 shows the same correlations, but only for genotyped bulls.

Table 2: Pearson correlations between (G)EBV_ss with MACE EBV and EBV_SENT, for local genotyped MACE sires, with or without any local information sent to INTERBULL, for milk MY), fat (FY) and protein yields (PY).

		MACE EBV (N=2828)	EBV_SENT (N=1036)
MY	EBV_ss	0.965	0.961
	GEBV_ss	0.973	0.956
FY	EBV_ss	0.951	0.961
	GEBV_ss	0.967	0.955
PY	EBV_ss	0.979	0.967
	GEBV_ss	0.979	0.962

The developed approach enabled integration of MACE EBV into multi-trait random regression test-day evaluations. This is shown by correlations between MACE EBV and EBV_ss that range between 0.95 and 0.98, and by regression coefficients close to 1 (Figure 2).

The new ssGBLUP approach enabled multi-trait random regression test-day ssGBLUP with integration of MACE EBV. The impact of genomic correlations on the evaluation of genotyped bulls is shown by the Pearson correlations between MACE EBV and GEBV_ss, ranging from 0.945 to 0.980. Compared to EBV_ss, we noticed a very small augmentation of correlations when genomic information is integrated (Table 1), and this augmentation is more visible when only looking at genotyped bulls (Table 2). Additionally, Figure 3 shows again excellent R² values and regression coefficients close to 1 (therefore no bias) with inclusion of genomic information, indicating a perfectly valid integration. Figures for fat and protein yields are not displayed but show the same tendency.

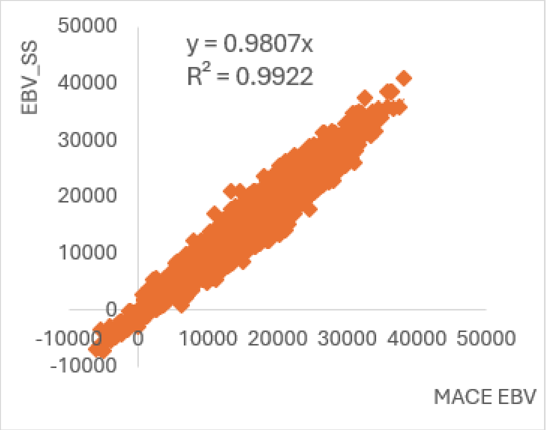

Figure 2. EBV_ss versus MACE EBV for all MACE bulls for milk yield (x10) expressed on the same scale

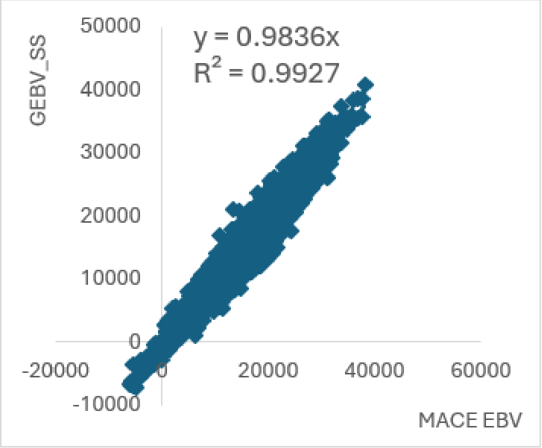

Figure 3. GEBV_ss versus MACE EBV for all MACE bulls for milk yield (x10) expressed on the same scales

Validation of the integration of genomic information

Walloon GEBV obtained from the current and developed genomic systems are similar. This is shown by Pearson correlations between GEBV calculated with the current and developed systems of 0.975 (fat yield), 0.984 (milk yield) and 0.986 (protein yield) for the 2828 genotyped MACE bulls. Additionally, Figure 4 shows again a very high R² value. Graphics for fat and protein yields are not displayed but show the same tendency.

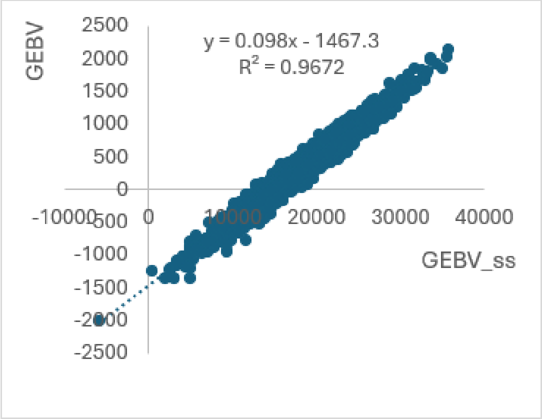

Figure 4. GEBV computed with the current (GEBV) and the developed (GEBV_ss) (x10) genomic system for all genotyped MACE bulls for milk yield, not expressed on the same scales.

Discussion

This study aimed to validate a single-step analysis that simultaneously incorporates all Walloon national data with available MACE information for milk production traits in Walloon Holstein dairy cattle. In recent years, ssGBLUP has become the preferred approach for genomic evaluation. Multiple countries are shifting their evaluations towards single-step evaluations, which has been reported to demonstrate less bias and higher accuracies, as proven by several countries in dairy cattle, (Zavadilova et al., 2014; Mäntysaari et al., 2020; Alkhoder et al, 2022; Himmelbauer et al., 2021; Pimentel et al. 2021, Cesarani et al., 2021); Guarini et al., 2019)) amongst others. Furthermore, studies in beef cattle (e.g., Lourenco et al. 2015, Bonifazi et al. 2023) have also demonstrated effectiveness. Our results show very high correlations and regression coefficients close to one, indicating good robustness and thus validating the method.

In the next step towards practical implementation, REL calculation will be performed according to the proposed strategy hereabove. This strategy has already been validated in our local methane evaluation (Chen et al., 2025) but has yet to be validated in the developed Walloon routine evaluation.

The deregression phase of MACE information is based on the quality of the parent averages, calculated directly by Interbull using a sire-grand-sire model. So, for a foreign bull with little or no information in Wallonia, the information is less reliable. However, as expected, as the number of daughters for this bull in Wallonia increases, most of the information in the bull proof comes from the daughters and/or sons (Mrode & Swanson, 1999). So, in the future, the quality of the DRP is expected to further improve.

Conclusions

As a conclusion, the proposed single-step GBLUP enabled an efficient genomic evaluation process, with robust validation statistics, highlighting its feasibility for routine use in Wallonia's genomic evaluations. The comparison between the current and developed genomic systems ensures that base change modifications of (G)EBV are acceptable, and that limited changes will be experienced by farmers. Future developments will continue to improve the evaluation system, such as for example a re-estimation of the variance components.

Acknowledgments

The authors would like to acknowledge the Walloon Breeders Association (Elevéo) for the data provision and endless support throughout the past years. Furthermore, we would like to thank the Walloon Region (SPW Wallonie) for its financial support, and ULiège for financing a travel grant.

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Introduction of single-step genomic evaluations in German Holsteins

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Abstract

In April 2025, we introduced single-step genomic evaluations for all traits that are subject to routine genetic evaluations in German Holstein. With all models, we estimate the same main effects as with the former conventional genetic evaluations of Holsteins. In addition, a fixed regression on the inbreeding coefficient was added to all the models. With the introduction of single-step models, reliabilities of GEBVs increased for all traits. This increase is especially pronounced in young animals with no own or offspring performance. As expected, the increase in reliability was greatest for many functional traits: longevity and direct calving index: +0.11, maternal calving index: +0.13, young stock survival and health index: +0.14, while it was lower for production traits: production index: +0.04. Additionally, validations with 2 and 4 years of right-truncated data confirm a substantial increase in the predictive ability of genomic GEBVs compared to the previous multi-step model: correlations of purely genomic GEBVs of young bulls with their later daughter-proven GEBVs are higher for all traits with the single-step model. Again, this increase in predictive ability is highest for the functional traits and lower for the production traits. With publication dates in April, August, and December, we conduct main runs with updated phenotypic information three times a year. In these main runs, we include MACE information from the respective current Interbull MACE run. In addition to these full runs, we conduct weekly genomic evaluations, for which we use the estimates of the SNP-effects and the residual polygenic effects from the main runs and apply them to the newly genotyped animals.

Key words: single-step model, genomic evaluation, genomic validation, Holstein cattle

Introduction

A multi-step SNP BLUP genomic model (MSM, Liu et al. 2011) was used for routine genomic evaluations of German Holsteins from August 2010 to December 2024. After the first publications of single-step genomic models (Aguilar et al. 2010; Christensen and Lund 2010), tremendous efforts were devoted to research and development in Germany with the goal of implementing a single-step SNP BLUP model (SSM, Liu et al. 2014) for routine genomic evaluations in German Holsteins.

There are a total of 10 trait groups evaluated routinely for German Holsteins. Logically, the SSM model must be implemented to all the trait groups simultaneously, to maintain the current weights of individual traits on total merit indices such as German RZG, RZ€ and RZOeko. To minimize the impact of genomic model change on genomic selection, all functionalities and features of the current MSM conventional and genomic evaluations must be retained as much as possible.

Although the conventional evaluations in MSM were multi-trait models for all trait groups, the genomic evaluations were single-trait models applied to deregressed conventional EBV (DRP) of reference bulls and cows (Liu et al. 2011). For instance, a multi-lactation random regression model was used to analyze test-day milk yields in the conventional evaluation of German Holsteins (Liu et al. 2004), but the evaluated trait in the genomic model of MSM was 305-day lactation milk yields combined over the first 3 lactations, calculated as a linear function of the genetic random regression coefficients from the conventional test-day model. In contrast to the MSM, SSM implicates direct modeling of the genomic information based on the national test-day milk yields. With the SSM, we therefore estimate for the first time SNP effects directly on the level of test-day random regression coefficients for production traits (Alkhoder et al. 2022; Alkhoder et al. 2024).

To enhance the reliability of genomic estimated breeding values (GEBV) and to represent foreign genetics in the German population as unbiased as possible, genotyped foreign bulls were included in the German Holstein genomic evaluation under the MSM model. The trait definitions of all traits evaluated in the Holstein MACE evaluation were used for the German genomic evaluation, e.g., the combined lactation 305-day milk yield under the MSM model as described above. With the introduction of the SSM model, phenotypic data of foreign cows is also included as in MSM model, via the MACE proofs of their sires, which increases the reliability of genomic prediction. In the SSM model, deregressed MACE EBVs of foreign bulls are treated as the same trait or a correlated trait as the national estimation traits, depending on the trait groups.

The objectives of this study were 1) to describe technical details of the genomic model upgrade from the MSM to the SSM model; and 2) to compare accuracy and prediction bias of the two genomic models via genomic validation.

Materials and Methods

Data sets for routine single-step evaluations

Phenotype data of cows and bulls

All national phenotypic data as used in the previous conventional evaluations are now used in SSM: phenotypic cow data recorded since 01.01.2000. As in the previous conventional and genomic MSM models, cows or calves with their own phenotypic records must have their sire and maternal grandsire known. The breeds of sire and maternal grandsire must be consistent with that of the animal. Other plausibility checks and edits on the data are also kept with SSM as they were for the conventional model in MSM. Because of the integration of foreign MACE data in the single-step evaluation, bulls with foreign daughter information in MACE are required to be born in 1995 or later to be consistent with the left truncation of national phenotype data.

Genotype data

Unlike the previous MSM model, genotype records of animals born before 2005 are no longer used in the SSM model to avoid possible negative impact of selective genotyping in the early years of genomic selection. Genotype imputing and routine checks on the genotype data are kept with SSM.

Pedigree data

In contrast to the trait-dependent pedigree processing procedures in the previous MSM evaluations, we now apply the same procedures to the sub-pedigrees for all trait groups: starting from youngest genotyped animals (including embryos) or cows / calves with phenotype records, a maximum of 20 generations of ancestors is traced back in the main pedigree. Additionally, at least 3 generations of ancestors are included for the oldest animals with phenotypic data, e.g., bulls with foreign daughters. Pedigree-based inbreeding coefficients are computed once, using all animals present in Germany's pedigree database for dairy breeds and the resulting inbreeding coefficients are then used to build the diagonals of the inverse relationship matrix and to define the fixed effect on the inbreeding coefficient in all SSM evaluations.

Data sets for two genomic validation studies

For a comparison between SSM and MSM, two comprehensive genomic validation studies were performed. For a 2-year validation, phenotypic data from the most recent two years were removed from the full evaluation 2304 to simulate an earlier evaluation in April 2021 (2104). The phenotypic, genotypic and pedigree data for the two evaluations 2304 and 2104 were already described in Tables 1 and 2 in the paper (Liu et al. 2023). Corresponding to the two different national cow data sets in evaluations 2304 and 2104, bull MACE data were obtained from the MACE evaluations in April 2023 and 2021.

In addition to this 2-year validation, a 4-year validation was conducted: with the results of a truncated conventional evaluation based on data as of the August 2020 evaluation (2008), Germany had also participated in Truncated MACE (TMACE) in October 2024. These TMACE EBVs were then considered in a subsequent, reduced SSM evaluation 2008, simulating an earlier SSM evaluation as of August 2020. Table 1 describes the data used for the full SSM evaluation 2408 and the reduced SSM evaluation 2008 for four test-day traits.

A genomic validation was conducted for all evaluated traits of German Holsteins, including MACE traits as well as national-only traits with both the 2-year and the 4-year validation data sets. For the earlier validation with 2304 and 2104 data, a linear regression test (LR, Legarra and Reverter, 2018) was applied. Using Interbull's GEBV Test software made available in September 2024, we conducted genomic validation with the second validation data set 2408 versus 2008.

Trait-wise development of SSM

Until today, a total of 10 trait groups are considered in routine evaluations of German Holsteins using: a multi-lactation random regression test-day model for milk, fat and protein yields and somatic cell scores (TDMS), a multi-trait model for 25 conformation traits (CONF), a multi-trait linear animal model for direct functional longevity (LONG), a multi-trait model for six female fertility traits (FERT), a multi-parity maternal-effect model for calving ease and stillbirth (CALV), a multi-trait model for workability traits (WORK), a multi-trait model for 16 direct health traits (HEAL), a multi-trait linear model for calf fitness (CFIT), a multi-trait random regression model for feed efficiency (FEFS), and a multi-trait model for four disposal reasons (DPRS) that serve as indicator traits for the health traits only.

Table 1. Description of the data sets for the single-step full and truncated evaluations of test-day milk, fat and protein yields and somatic cell scores

	Single-step evaluation	
Frequency	2408	2008
Genotyped animals	1,631,844 Holstein animals (1,433,599 females and 198,245 males)	
Phenotyped cows or bulls	14,189,574	12,195,546
Test-day records	277,884,084	235,578,132
Genotyped or phenotyped animals	15,165,965	13,565,673
Animals in pedigree SSM	22,743,486	20,983,007
Reference animals MSM	604,587	246,910

Starting with the simplest evaluation model for the conformation trait group CONF with only 1 record per cow, we tested the SSM for the genomic evaluation of German Holsteins (Alkhoder et al. 2021). After a positive experience with the simplest statistical model, we extended the SSM test implementation to the two most complex statistical models: the multi-lactation random regression test-day model for milk production traits and somatic cell scores TDMS (Alkhoder et al. 2022) and the multi-parity maternal-effect model for calving ease and stillbirth CALV (Alkhoder et al. 2022a). Having successfully tested the SSM model for the three trait groups CONF, TDMS and CALV, we finally moved on to include all the remaining 7 trait groups and completed the SSM testing processes in 2023.

Integration of bull MACE data

Due to the simplicity of this approach, the previous conventional evaluations from MSM are still run in parallel to SSM at every main run (3x per year) to provide genomic-free EBVs to Interbull as input for MACE.

The current Interbull MACE evaluation uses a single-trait multi-country model. For instance, the MACE trait of milk yield for German Holstein is 305-day lactation milk yield combined over the first three lactations, which corresponds to an aggregated linear function of the 9 random regression coefficients used in the German random regression test-day model (Liu et al. 2004; Alkhoder et al. 2024). Unlike the MSM genomic evaluation that was previously applied to DRP from the aggregated, combined traits on lactation basis, the SSM model analyzes original cow test-day milk yields also on the genomic level. Another example is the calving trait model: a multi-parity maternal-effect single-step model for calving ease and stillbirth phenotypic records of calving cows in the first three parities. In comparison, DRP of first parity calving ease or stillbirth, defined as official breeding values for publication, were pseudo-phenotypic records for the single-trait MSM evaluation.

Across all the trait groups, the trait definition for the single-trait MSM model was the German official breeding value for publication as well as the MACE trait, some of which were aggregated from the original national traits / estimation variables.

Deregressed MACE EBVs are included in the SSM for the different trait groups as correlated traits, using the national genetic covariance matrices and the weights used to combine national traits to MACE traits to derive covariances between national and MACE traits. In most cases, the genetic correlation between the combination of national traits and the MACE trait is assumed to be 1. Only in some cases, this correlation was pruned to 0.97 to enhance convergence.

Calculation of indices from the SSM

After completion of the test implementations for all the 10 trait groups, we upgraded our calculation procedures for various sub-indices, and the German total merit indices *RZG* and *RZ€* for the SSM system slightly.

The previously used non-linear index for longevity (RZN, Heise 2017; Heise et al. 2016) was replaced with its linear approximation and we adapted the genetic standard deviation that is used to express the production index RZM on its relative scale, resulting in a reduction of variance of GEBVs for RZM. The procedures to calculate all other index breeding values are retained from MSM.

Modelling inbreeding depression in the SSM

Pedigree-based inbreeding was considered in the previous MSM evaluations only with its effect on the pedigree-based relationship matrix for German Holstein evaluations. And the effects of inbreeding depression were ignored. With SSM, we now include a fixed effect in form of a linear regression on the pedigree-based inbreeding coefficient for all traits.

Using sire genotypes for calf fitness

An unexpectedly high genetic trend was observed in the initial SSM developments for the early-measured calf fitness CFIT (young stock survival). Causes for the overestimated SSM GEBVs were traced back to the delayed genotyping of female calves and the therefore extremely limited genotyping of dead or sick calves for the early survival traits (Alkhoder et al. 2025). As a validated solution to the inflated genomic prediction, we use only genotypes of the sires of the female calves. This contrasts with all other trait groups, where we use genotype data of all animals born 2005 or later.

Weekly evaluations with the SSM

In addition to the full SSM evaluations, a weekly genomic evaluation system was developed (Alkhoder et al. 2004). From the SSM evaluation that includes the current MACE data, SNP effect estimates and residual polygenic effect estimates for all the genotyped animals are obtained and then applied to the newly genotyped animals weekly. Direct genomic values of the new animals are computed using the SNP effect estimates and allele frequencies of SNP markers. Residual polygenic effects and GEBVs of the newly genotyped animals are indirectly predicted based on their pedigree relationship to the genotyped animals that are included in the main evaluation.

Parallel SSM genomic evaluations in the testing phase

The introduction of SSM represented a significant improvement over the previous MSM genomic evaluation and caused greater changes to GEBVs of many animals, especially the youngest genotyped animals. This imposed a challenge to breeding organizations and farmers for adopting to SSM. To help the users of genomic evaluations adapt to the new SSM model smoothly, we conducted parallel SSM evaluations in the testing phase. Starting with the August 2024 main evaluation, SSM test evaluation results were provided to the breeding organizations for the purpose of receiving valuable feedback from the breeders' perspective to further optimize all SSM systems and models. After the development and internal testing of the SSM weekly evaluation system (Alkhoder et al. 2024), we also provided the breeding organizations with test results from weekly evaluations starting in October 2024. In multiple workshops with various delegates of the breeding organizations, we gathered the feedback and used it to 1) answer yet open questions from the breeders, 2) to further improve our communication strategies regarding the introduction of SSM, and 3) to further improve our SSM models and procedures.

Results and Discussion

SSM evaluations for all the 10 trait groups are conducted using the software MiX99 (Strandén and Lidauer,1999). Approximate genomic reliabilities for GEBVs from the SSM are computed using either APAX99 from the MiX99 software suite or using own Fortran programs (Liu et al. 2023), following Interbull's Guidelines for approximating genomic reliabilities for SSM (Liu et al. 2024). Similarly, effective daughter contributions (EDC) of bulls and effective record contributions (ERC) of cows or calves are calculated using either APAX99 or own programs. The sub-indices of individual trait groups as well as the total merit indices RZG and RZ€ are computed with a central, configurable software, developed in Python. All workflows are managed with Snakemake as a workflow management software (https://snakemake.readthedocs.io/en/stable/project_info/citations.html).

Differences in technical steps between the SSM and MSM models are schematically described in Figure 1. The SSM model simplifies the genomic evaluation process with a joint analysis of genotyped and non-genotyped animals having or having no phenotypic data in one single step. In contrast, genotype data was used at a later stage of the evaluation process for MSM than phenotype data.

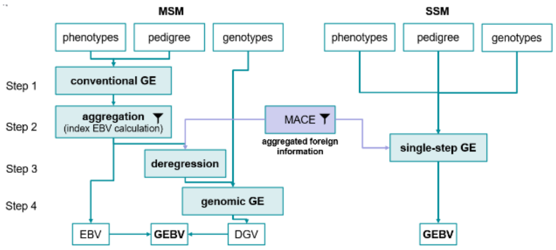

Figure 1. Comparison of technical steps between the single-step and multi-step genomic models

Figure 2 shows the numbers of validation bulls used in both validation studies: 2408 vs 2008 and 2304 vs 2104. The selection of validation bulls was conducted, following the Interbull GEBV test rules (Mäntysaari et al. 2010) for the validation study 2408 vs 2008. However, an additional selection criterion was imposed on the validation bulls for the earlier validation 2304 vs 2104: the validation bull had to be owned by a German AI organization. Unlike the 4-year validation 2408 vs 2008, the 2-year validation 2304 vs 2103 did not include data from TMACE. Instead, the actual MACE results from 2104 were used, and thus the results of the validation may likely also be impacted by model changes between 2104 and 2304 in Germany or in the other countries participating in MACE. Overall, the numbers of validation bulls are reasonably high to deduce reliable and accurate validation results.

Figure 3 shows, based on the 2-year validation 2304 vs 2104, GEBV correlations of the validation bulls between the early evaluation 2104 as young candidates without daughters and the later evaluation 2304 when these bulls were evaluated as daughter-proven bulls. The SSM model has evidently higher GEBV correlation, i.e., prediction accuracy, than the MSM model for all traits, RZG and RZ€. Similar levels of prediction accuracy are also observed for the validation study 2408 vs 2008.

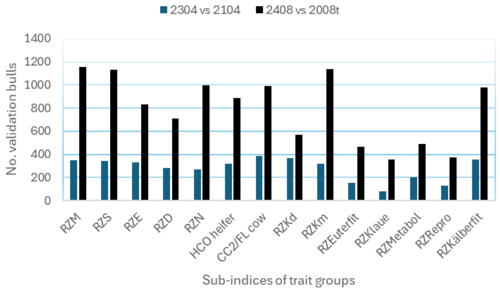

Figure 2. Number of validation bulls for all trait groups in the two validation studies

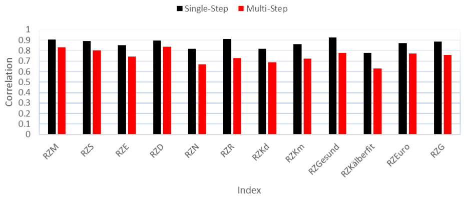

Figure 3. GEBV correlations of the validation bulls between two evaluations 2304 and 2104 (2-year validation) for the indices

For a total of 258 German AI bulls born in 2021, we compared their genomic reliabilities between SSM and MSM in the evaluation as of April 2023. Figure 4 shows the increase of genomic reliabilities from MSM to SSM for all the trait group indices, RZG and RZ€. The functional traits, like direct health traits (RZGesund) and calf fitness (RZKälberfit), gain more in reliabilities than the production trait index RZM. The traits which already had high reliabilities under the MSM tend to have a smaller increase in reliabilities when upgraded to the SSM model, such as somatic cell scores RZS.

Prediction bias, measured as the regression slope from the two validation studies, is given in Table 2. As mentioned above, Interbull's official GEBV test software was used for the validation 2408 vs 2008, with deregressed GEBVs as the dependent target variable. However, for the 2-year validation study 2304 vs 2104, our own software for a weighted linear regression (Legarra and Reverter 2018) was applied to the two SSM and MSM models.

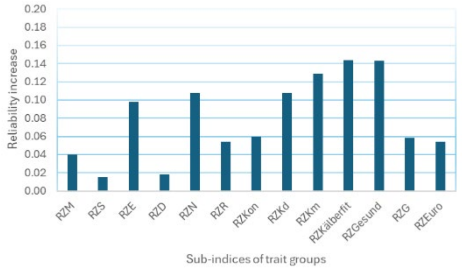

Figure 4. Increase of the genomic reliability from the multi-step to single-step model for German AI bulls in the 2-year validation

Across all the trait groups and both validation studies, we can see that both SSM and MSM give nearly unbiased prediction for almost all the trait groups, but the SSM has somewhat less over- or under-estimation, i.e., b_1 is closer to 1, than the MSM model. Using the deregressed GEBVs as dependent variable of the GEBV test results in b_1 values deviating more from 1 than using direct GEBVs as dependent variable, verified for the new calf fitness SSM model (Alkhoder et al. 2025). Over all traits of the 10 trait groups evaluated for German Holstein, we can see that neither overestimation nor underestimation seem to be an issue for the genomic models in German Holsteins. Relatively poor b_1 estimates were obtained for the calving traits, which may possibly be explained by the fact that very few stillborn/dead calves are usually genotyped. Additionally, male calves that have more problems with calving ease or stillbirth than female calves are rarely genotyped, in contrast to female calves that are all genotyped under the German whole-herd genotyping scheme if the herd participates in genotyping. Because of the relatively short history of routine recording of direct health traits and some new conformation traits in German Holsteins, the removal of phenotypic data from the last four years for the validation 2408 vs 2008t reduces the representativeness of the validation results for these traits, i.e., caution needs to be taken when interpreting the b_1 estimates for the direct health traits in the validation study 2408 vs 2008. No genomic validation was conducted for feed efficiency due to the small number of genotyped cows with dry matter intake records and a lack of cow sires that qualified for being selected as validation bulls.

Figure 5 shows GEBV correlations for the RZG between SSM and MSM for 8,661 genotyped German Holstein Black-and-White AI bulls born between 2005 and 2023 using the evaluation results as of December 2024. The GEBV correlation for AI bulls with daughters is about 0.95 and drops to 0.85 for the youngest purely genomic AI bulls born in 2023, although GEBV correlations for the youngest AI bulls are about 0.95 for individual traits like milk production and conformation traits (data not shown here). The lower RZG correlation is caused, besides the model changes, also by the changes introduced in the milk production index RZM and the longevity index RZN.

Table 2: Regression slope estimates of the GEBV test of selected traits for the genomic models in the two validation studies

Trait	2408 vs 2008	2304 vs 2104	
	SSM	SSM	MSM
Milk yield	1.02	1.02	1.01
Fat yield	1.06	1.03	1.15
Protein yield	1.05	1.03	1.10
Somatic cell scores	1.11	1.05	1.07
Functional longevity	0.97	1.00	0.95
Heifer fertility HCO	1.17	1.05	1.11
Cow fertility CC2	0.98	0.99	0.88
Stillbirth direct	0.84	0.88	0.97
Calving ease maternal	0.78	0.89	0.96
Milking speed	1.01	1.06	1.13
Milking temperament	0.97	0.96	0.97
Stature	0.99	1.03	1.07
Udder support	0.98	1.01	1.23
Body condition score	1.07	1.10	1.09
Locomotion	1.01	1.00	1.14
Digital dermatitis	0.88	0.92	0.92
Clinical mastitis	0.58	0.86	0.67
Calf fitness	0.95	1.04	0.92

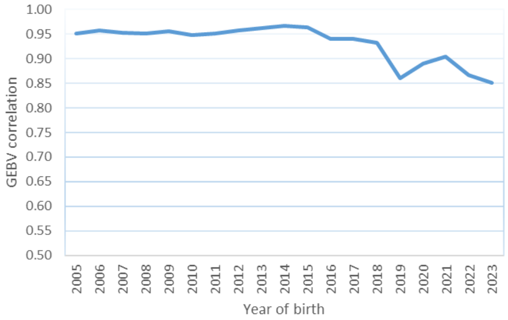

Figure 5. GEBV correlations of the total merit index RZG between SSM and MSM for German Holstein AI bulls

Like Figure 5 for AI bulls, Figure 6 shows GEBV correlations of RZG for 1,478,613 genotyped Black-and-White Holstein female animals. For all the female animals born from 2016 on and genotyped under the German whole-herd genotyping scheme, SSM and MSM have a GEBV correlation of about 0.95 for the total merit index RZG, despite the above-mentioned additional changes impacting

RZG. The youngest female animals have much higher RZG GEBV correlation than the youngest AI bulls, 0.95 vs 0.85, which may be attributed to the different selection intensities between the two groups of genotyped animals.

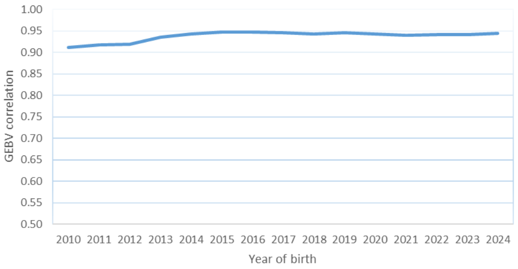

Figure 6. GEBV correlations of the total merit index RZG between SSM and MSM for genotyped Holstein female animals

With respect to expectation management during the preparations prior to the introduction of SSM, we emphasized clearly from the beginning that the change to SSM was a major upgrade of the genomic model and would thus have a profound impact on the breeding organizations' selection programs. In addition to the early research projects on the theory of the SSM model, we invested huge efforts and resources to develop the new evaluation systems as well as to help the breeding organizations and farmers adjust their programs for the new SSM model. Besides the numerous parallel full and weekly SSM test evaluations, we provided our new results at several meetings with our customers and improved our SSM systems based on their feedback. These iterations of providing additional analyses and explanations to our breeding organizations and including customer feedback in the improvement of our models led to a strong support of the introduction of SSM by the German breeding organizations.

As the MSM genomic model uses deregressed proofs as the dependent variable / pseudo-records calculated from the conventional evaluation which ignores the rapid genetic progress by genomic selection, the differences between the two genomic models SSM and MSM are likely to increase with time. A delayed update from MSM to SSM would make it increasingly difficult for farmers and breeders to adapt to the one-time model change.

Conclusions

SSM represents a major improvement of our genomic evaluation compared to the previous MSM. All genotypic, phenotypic and pedigree data are jointly evaluated by the single-step model, resulting in a higher accuracy of the genomic prediction. With two genomic validation studies, we have shown higher prediction accuracy, i.e., correlation between the early candidate GEBVs and later daughter-proven GEBVs for AI bulls and higher genomic reliabilities for all traits from SSM compared to MSM. GEBVs from SSM have been proven to be more stable across evaluation runs and to have substantially increased reliabilities compared to GEBVs from the previous MSM for all the trait groups. Because of these major improvements, the introduction of SSM received strong support from the breeders and their breeding organizations, despite the relatively large one-time changes. Intensive collaboration with the German breeding organizations prior to and after the introduction of SSM led to strong commitment from the breeders' community and helped the introduction of SSM in German Holsteins become a great success.

Acknowledgments

We thank our colleagues from the German breeding organizations who supported us with their honest feedback and their suggestions for improvements without which the introduction of SSM in German Holsteins would not have become the success that it was.

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Transition of the UK dairy national evaluation to across-breed and single-step genomic evaluation: somatic cell counts as a case trait

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Abstract

Current UK genomic evolutions follow a two-step approach; initially, a genetic evaluation based solely on pedigree information, followed by a Single Nucleotide Polymorphism Best Linear Unbiased Prediction (SNPBLUP) analysis for genotyped animals using de-regressed proofs, including MACE proofs from Interbull. Nowadays, with recent advances in computational feasibility and growing interest in across-breed genomic evaluations for dairy herds, there is a compelling need to adopt a single-step across-breed genomic evaluation approach within the UK dairy industry. The single-step method offers notable advantages by simultaneously incorporating genotypes and both recent and historical pedigree and phenotypic data into a single analysis. This integration enhances the accuracy of genetic predictions across diverse breeds, accelerates genetic progress, and improves selection efficiency. This study aims to evaluate the impact of using genomic information and compare the prediction ability of single-step genomic evaluation (using ssSNPBLUP method) and pedigree-based genetic evaluation (PedBLUP) employing cross-validation techniques (Linear Regression method). The trait analysed was somatic cell count (SCC), using data from the UK national evaluations as of the December 2024 official run. The dataset included 11,271,959 animals in the pedigree and 19,056,954 SCC records from 7,527,712 cows. Foreign information was incorporated for 182,844 bulls, with adjustments made to avoid double counting of domestic data. Genotypic data was available for 891,480 animals, imputed at 79,051 SNPs using findhap.f90 V3. Analyses were performed using the MiX99 V23.1026 software, applying an ssSNPBLUP model with 10% polygenic effects. The validation group comprises bulls born after 2016 and cows born after 2018, whose records are set to missing. Results showed a genomic accuracy improvement of up to 54% in cows when comparing ssSNPBLUP to PedBLUP. Among bulls, the greatest gain was observed in Holsteins (+33%), followed by Guernsey and Ayrshire (+30%), and Jersey (+20%). Level bias and dispersion bias was slightly reduced in ssSNPBLUP relative to PedBLUP. Overall, the findings demonstrate that single-step genomic evaluation is a promising and efficient approach for enhancing prediction accuracy in UK dairy cattle.

Key words: single-step genomic evaluation, accuracy, cross-validation, UK dairy industry

Introduction

The genetic evaluation in the UK dairy sector is undergoing a significant transition. Currently, genetic evaluation has relied on a two-step method. This includes conventional pedigree-based evaluations followed by a genomic prediction step using Single Nucleotide Polymorphism Best Linear Unbiased Prediction (SNPBLUP), incorporating de-regressed proofs from Interbull for foreign bulls. Recent advances in computational capacity, as well as the growing interest in across-breed genomic evaluations for dairy herds, there is a compelling need to adopt a single-step across-breed genomic evaluation approach (ssGBLUP) within the UK dairy industry. This approach simultaneously integrates pedigree, phenotypic, and genomic information into a single evaluation model, offering numerous advantages: inclusion of all available data, improving prediction accuracy, mitigate the effects of preselection bias associated with multi-step approach, and, consequently, accelerating the genetic progress.

This report focuses on somatic cell count (SCC) as a trait study to evaluate the prediction ability of ssGBLUP in comparison to traditional pedigree-based BLUP (PedBLUP) employing cross-validation techniques (Linear Regression method).

Materials and Methods

Data

Somatic cell count data was obtained from the UK national evaluations as of the December 2024 official run. The dataset included 11,271,959 animals in the pedigree and 19,056,954 SCC lactation records from 7,527,712 cows. Foreign information was incorporated for 182,844 bulls, with adjustments made to avoid double counting of domestic data. Genotypic data was available for 891,480 animals, imputed at 79,051 SNPs using findhap.f90 V3. This reference imputation panel was derived by USDA (Al-Khudhair et al., 2021).

Model and analyses

SCC was analyzed as $\log_e$ SCC and the model included herd-year-season, lactation, age nested within parity, month of calving as fixed effects and random effects of herd-sire interaction, permanent environment and animal. A pedigree of five generations was used in the analysis and UPGs were used for missing parents based on year of birth, breed, country of origin and the four paths of dam of cows and bulls, and sire of bulls and cows.

MACE proofs were included following Bonifazi et al. (2023). First, effective record contribution (ERC) was derived from the reliability of the animal ($\mathrm{ERC}=\lambda\cdot($ reliability/(1-reliability)); where $\lambda=(1-$heritability)/heritability). In second, the de-regressed proofs (DRP) were calculated as follow: $\mathrm{DRP}=\mathrm{PA}+(\mathrm{EBV}-\mathrm{PA})/(\mathrm{dERC}/(\mathrm{dERC}+\lambda))$, where PA is the parent average and $\mathrm{dERC}=\mathrm{ERC}-\mathrm{ERC}_{\mathrm{PA}}$. To avoid double counting of the national data (NAT) of bulls with UK daughters from interbull (international, INT) proofs, DRP were blended as follow: $\mathrm{DRP}^{*}=((\mathrm{dERC}_{\mathrm{INT}}\cdot\mathrm{DRP}_{\mathrm{INT}})-(\mathrm{dERC}_{\mathrm{NAT}}\cdot\mathrm{DRP}_{\mathrm{NAT}}))/\mathrm{dERC}^{*}$ where $\mathrm{dERC}^{*}=\mathrm{dERC}_{\mathrm{INT}}-\mathrm{dERC}_{\mathrm{NAT}}$. These de-regressed proofs were used as pseudo-records with the corresponding ERC as weights in the model.

Analyses were performed using the MiX99 V23.1026 software (Strandén et al., 2017), applying a ssSNPBLUP (Liu et al., 2014) model with 10% polygenic effects.

Validation

A cross-validation technique, linear regression method, was used to compare ssGBLUP and PedBLUP following Legarra & Reverter (2018). The different estimators' statistics of bias (difference of means), dispersion (slope of the regression) and accuracy were calculated based on partial and full runs. The validation group included bulls born after 2016 and cows after 2018. The phenotypes of these cows together with the de-regressed proofs of these bulls were excluded from the "partial" runs.

Results & Discussion

The implementation of the single-step genomic BLUP model led to substantial improvements in prediction accuracy over the traditional pedigree-based BLUP (PedBLUP), with gains observed across all breeds: Guernsey, Holstein, Jersey, and Ayrshire. In cows, prediction accuracy improved dramatically, with Holstein showing the largest relative gain of +102% (accuracy increased from 0.32 to 0.648). Guernsey and Ayrshire cows followed with increases of +65% and +56%, respectively, while Jersey cows exhibited a +43% improvement, but notably had the 2nd highest ssGBLUP accuracy. Bulls also benefitted from the use of ssGBLUP, with Holstein bulls achieving the highest accuracy gain of +33%, followed by Guernsey and Ayrshire (+30%), and Jersey (+20%). The advantage of ssGBLUP is coming from leverages genomic information to capture Mendelian sampling more effectively than PedBLUP. The improved prediction accuracies of ssGBLUP in both sexes are summarized in Tables 1 (bulls) and 2 (cows).

Level bias, as illustrated in Figure 1, was marginally reduced under ssGBLUP relative to PedBLUP across all breeds and both sexes. For example, the level bias for Jersey bulls was -0.001 and -0.056 for both ssGBLUP and PedBLUP, respectively, reducing the level bias to mostly null when using ssGBLUP. This reduction indicates that genetic evaluations using ssGBLUP are more centered on the true genetic values, thus improving the reliability of selection. Although the magnitude of the reduction was modest generally across breeds and sexes, it consistently favored ssGBLUP and supports its use for less biased evaluations.

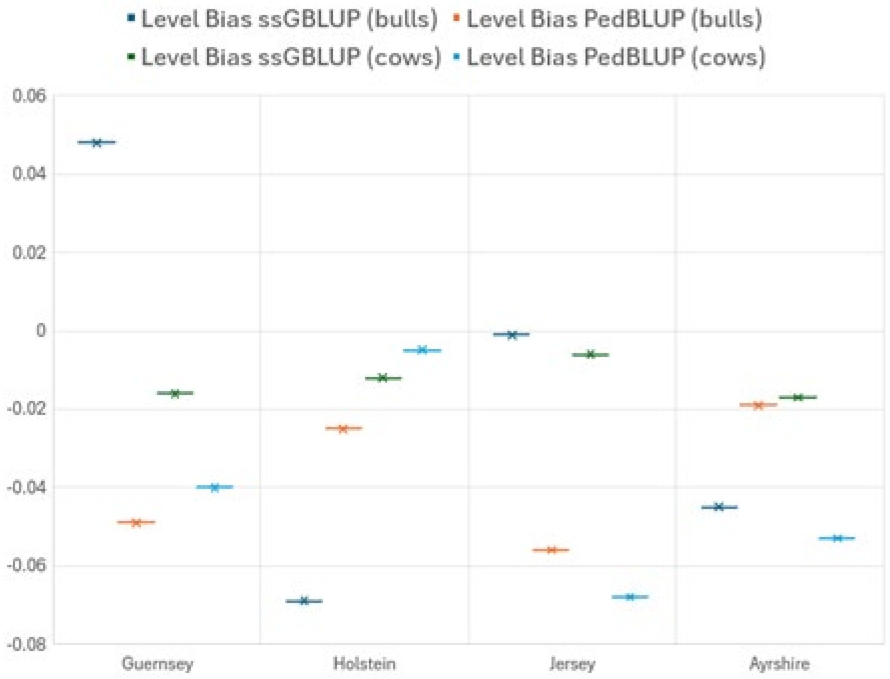

Figure 1. Level Bias for single-step genomic evaluation approach (ssGBLUP) and pedigree-based BLUP (PedBLUP) accords breeds.

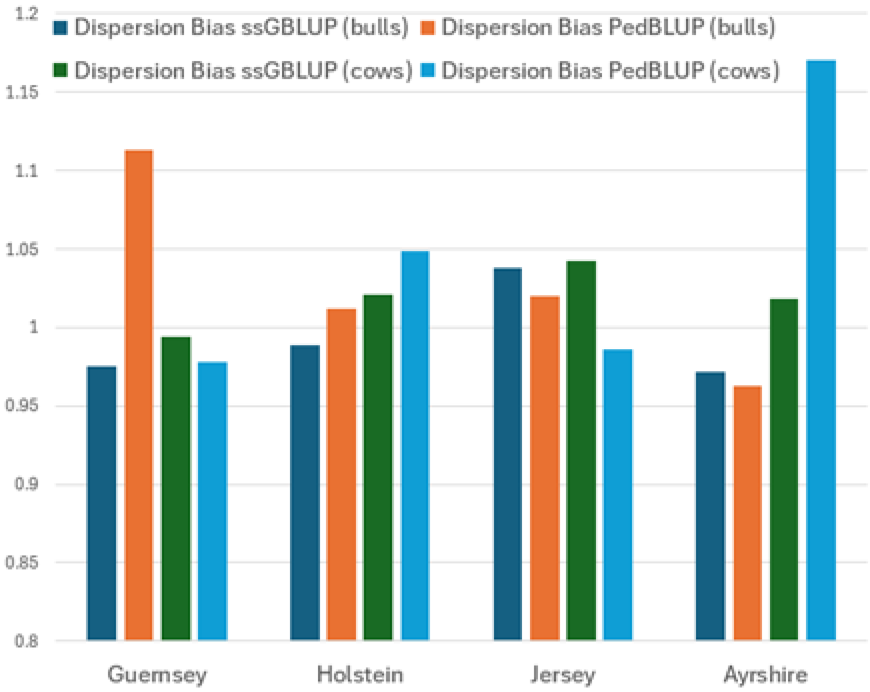

Figure 2. Dispersion Bias for single-step genomic evaluation approach (ssGBLUP) and pedigree-based BLUP (PedBLUP) accords breeds.

On the other hand, as shown in Figure 2, ssGBLUP consistently produced dispersion bias values closer 1.00. In contrast, PedBLUP tended to over-dispersion in several cases, for example, Ayrshire cows displayed a dispersion bias of 1.17 versus 1.02 for ssGBLUP. Also, Guernsey bulls presented a dispersion bias of 1.11 in comparison to 0.975 for ssGBLUP. Overall, ssGBLUP showed more balanced dispersion across all breeds and sexes, particularly improving prediction spread in cows.

Additionally, within breed ssGBLUP were performed which in all cases yielded lower accuracies compared to the across breed ssGBLUP (results not shown in this paper).

These results highlight ssGBLUP's ability to provide more reliable genetic evaluations.

Table 1: Comparison of prediction ability in bulls.

Breed	Number of animals	Accuracy ssGBLUP	Accuracy PedBLUP	Increase % ACC	Correl Full PedBLUP # Partial ssGBLUP
Gue	28	0.50	0.38	29.2	0.50
Hol	6,087	0.79	0.69	13.9	0.90
Jer	453	0.61	0.57	8.3	0.87
Ayr	245	0.51	0.44	14.9	0.79

*Gue: Guernsey; Hol: Holstein; Jer: Jersey; Ayr: Ayrshire.

Table 2: Comparison of prediction ability in cows.

Breed	Number of animals	Accuracy ssGBLUP	Accuracy PedBLUP	Increase % ACC	Correl Full PedBLUP # Partial ssGBLUP
Gue	855	0.45	0.27	65.1	0.40
Hol	95,965	0.65	0.32	102.2	0.60
Jer	3,512	0.50	0.35	43.1	0.60
Ayr	382	0.42	0.27	56.1	0.42

*Gue: Guernsey; Hol: Holstein; Jer: Jersey; Ayr: Ayrshire.

Conclusions

The cross-validation results clearly demonstrate that single-step genomic evaluation is a more effective evaluation method for predicting genetic merit, particularly in genotyped animals. The results support the adoption of a unified Single-Step Genomic BLUP framework for genetic evaluation in UK dairy cattle. By combining genomic, pedigree, and phenotypic information in a single model, ssGBLUP improves prediction accuracy, increases selection efficiency, and accelerates genetic gain across breeds. A national implementation would simplify evaluations, allow the evaluation of crossbreed animals, and help ensure a more competitive and sustainable breeding program.

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One *Moo-ve* Closer: Single-Step Genomic Predictions for Crossbred Holstein and Jersey Cattle Using Metafounders

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Abstract

The study examined the impact of incorporating metafounders (MF) in single-step genomic BLUP (ssGBLUP) models for the genetic evaluation of Holstein (HO) and Jersey (JE) cattle with their crossbreds (CROSS). The dataset included 23,736,975 records on 8,560,986 cows. Genotypic data on 181,379 JE, 1,905,292 HO, and 53,799 CROSS animals was used for the evaluation. The genetic evaluation included five production traits, namely milk yield (MY), protein yield (PY), fat yield (FY), somatic cell score (SCS), and daughter pregnancy rate (DPR), which were analyzed using a five-trait repeatability model using ssGBLUP with or without MF. Three different MF scenarios were tested: 4MF (based on breed), 24MF (based on the combination of breed, sex, and year of birth), and 32MF (similar to 24MF but with CROSS as a separate genetic group). The three MF scenarios were compared to a conventional ssGBLUP model that did not include metafounders (NO_MF). Forward-in-time validation was carried out to evaluate predictability, inflation, and stability. For purebred Holstein and Jersey cows, the truncated dataset included phenotypes through December 2018, whereas for crossbreds the cutoff was December 2015; the complete dataset extended through December 2022. Validation targeted genotyped cows lacking records in their respective truncated dataset but with at least one record in the complete dataset, yielding 96, 295 Holsteins 26, 436 Jerseys, and 5,099 crossbreds for analysis. Results showed that including MF affected prediction metrics differently depending on the trait, breed, and MF configuration. While certain MF classifications (e.g., 4MF) reduce bias and improved predictability in crossbreds for some traits, others showed minimal effects, particularly in purebred Holsteins. For low heritability traits (SCS, DPR), MF scenarios provided better predictive ability in CROSS animals. In contrast, for high heritability traits (MY, PY, FY), stability tended to decrease in MF models, suggesting possible overfitting due to added model complexity. Overall, MF offers a promising strategy to address pedigree gaps in multibreed evaluations, but its application should be carefully tailored to trait architecture and population composition to avoid overfitting and ensure accurate genetic predictions.

Key words: base population, genomic evaluation, metafounder, single-step genomic BLUP

Introduction

Traditionally, genomic evaluations for dairy cattle have been conducted on a single-breed basis, often excluding crossbred animals. However, the growing proportion of crossbreds in U.S. herds underscores the importance of including them in evaluations to improve management decisions. From 1990 to 2018, the proportion of crossbred cows in the U.S. Dairy Herd Improvement program rose from 0.1% to 5.3% (Guinan et al., 2019). Recognizing this trend, the Council on Dairy Cattle Breeding (CDCB) extended genomic evaluations to crossbred animals in 2019 (Wiggans et al., 2019; CDCB, 2020).

Several methods have been proposed for joint evaluations of purebred and crossbred animals (Wei & van der Werf, 1994; Christensen et al., 2014; Steyn et al., 2021; VanRaden et al., 2020). A straightforward approach combines all genotypes in a single relationship matrix (Lourenco et al., 2016). The single-step genomic BLUP (ssGBLUP) approach integrates pedigree (**A**) and genomic (**G**) matrices to estimate genomic breeding values (GEBV) (Aguilar et al., 2010; Christensen & Lund, 2010). However, ssGBLUP requires uniform scaling between **A** and **G** and a consistent base population (Christensen, 2012). Incomplete pedigrees and population stratification complicate these assumptions.

To address these issues, Thompson (1979) and Quaas (1988) introduced unknown parent groups (UPG) to account for missing pedigree information. More recently, Legarra et al. (2015) proposed metafounders (MF) to model relationships among base populations, improving compatibility between **A** and **G**. MF consider allele frequencies of 0.5 across markers and estimate relationships among pseudo-ancestors using a gamma matrix (**Γ**). Studies have shown that MF can improve prediction accuracy in multibreed populations (Garcia-Baccino et al., 2017; Xiang et al., 2016).

Despite these advances, limited work has assessed MF performance in combined Holstein-Jersey ssGBLUP models, particularly regarding crossbred evaluations. This study aims to evaluate different MF classifications and their effects on accuracy, bias, and stability in genomic predictions of purebreds and crossbreds.

Materials and Methods

Official data files from Zoetis Inc. were used for this study. Phenotypic and pedigree data were sourced from U.S. dairy producers via backups from herd management systems (DairyComp 305, PC Dart, and DHI Plus). Quality control excluded lactations with data collection ratings (DCR) <0.70 or implausible yields, and pedigree was traced back 20 generations where possible. Pedigree completeness varied: 57.2% of animals had known parents, 10.3% had missing sires, 8.6% had missing dams, and 23.8% had both parents unknown.

DNA was extracted and genotyped on Illumina BeadArray platforms (3K–80K SNPs). Low-density genotypes (<40K SNPs) were imputed to 45,245 markers using FImpute (Sargolzaei et al., 2011), achieving 97% concordance.

The genetic evaluation included five production traits: milk yield (MY), protein yield (PY), fat yield (FY), somatic cell score (SCS), and daughter pregnancy rate (DPR). Official records comprised 23.7 million observations on 8.56 million cows, with genotypes available for 1.91 million Holsteins (HO), 181,379 Jerseys (JE), and 53,799 crossbred (CROSS) animals. Table 1 summarises the total number of records and number of studied animals across traits defined by breed. Heritabilities (±SE) for the five traits were 0.35 (0.005) for MY, 0.29 (0.008) for FY, 0.31 (0.014) for PY, 0.13 (0.008) for SCS, and 0.07 (0.003) for DPR.

Table 1: number of records and cows with phenotypes and genotypes

Group	Phenotypes	Genotyped animals (ssGBLUP only)	
	N	Cows	
Holstein	20,166,782	7,298,374	1,905,292
Jersey	2,868,461	996,353	181,379
*CROSS	701,732	266,259	50,938
Total	23,736,975	8,560,986	2,137,609

*CROSS = Crossbred of Holstein x Jersey, N = Number of records, Cows = Number of cows with records

Genomic breed composition was determined using a supervised admixture model (Zoetis proprietary pipeline). Purebred HO and JE were defined as ≥80% ancestry; CROSS animals had combined HO and JE ancestry ≥80%. Three validation sets were created: 96,295 HO, 26,436 JE, and 5,099 CROSS cows. Reduced datasets included records until Dec 2018 (HO, JE) or Dec 2015 (CROSS); complete datasets extended to Dec 2022.

Models included five-trait repeatability with random animal, permanent environment, and herd × sire interaction effects, and fixed effects included contemporary groups, heterosis and inbreeding. The genomic relationship matrix (G) and pedigree matrix (A) were combined in a single-step GBLUP (ssGBLUP) using the Algorithm for Proven and Young (APY) (Legarra et al., 2009; Aguilar et al., 2010) with a random core size of 30,000: 22,156 females and 1,931 males for HO, 5,643 females and 181 males for Jersey, and 678 females for crossbred. Models were solved based on iteration on data with the preconditioned conjugate gradient (PCG) in algorithm BLUP90IOD2OMP1 (Tsuruta et al., 2001).

Forward-in-time validation assessed (1) predictability as the correlation between adjusted phenotypes and GEBVs. Adjusted phenotypes were obtained using PREDICTf90 v1.3 (Misztal et al., 2014); (2) inflation as the regression slope of phenotypes on GEBVs (ideal slope = 1); and (3) stability as the correlation between GEBVs estimated from reduced and complete datasets. Standard errors for predictabilities and stabilities were computed following Bermann et al. (2024). All regression and correlation analyses were performed in R software (R Development Core Team, 2024). The different models with and without MFs are detailed next.

SSgblup Analyses

All computations with ssGBLUP were done using the full pedigree with 27 million animals and the genomic relationship matrix for 2,137,609 animals. The ssGBLUP allows the creation of a joint relationship matrix for genotyped and non-genotyped animals by replacing the inverse of the pedigree relationship matrix, $\mathbf{A}^{-1}$, with the inverse of the **H** matrix that combines the pedigree (**A**) and the genomic relationship matrix **G** (Legarra et al., 2009; Aguilar et al., 2010):

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

where $\mathbf{A}^{-1}$ is an inverse of the pedigree relationship matrix; $\mathbf{G}^{-1}$ is an inverse of the genomic relationship matrix (VanRaden, 2008); and $\mathbf{A}_{22}^{-1}$ is an inverse of the pedigree relationship matrix for genotyped animals only.

Single-step GBLUP with metafounders

The $\mathbf{H}^{-1}$ matrix considers relationships among MF ($\mathbf{\Gamma}$) in the MF approach. Hence, it is replaced with the $(\mathbf{H}^{\Gamma})^{-1}$ matrix, as described by Legarra et al. (2015) and Christensen et al. (2014). In this way, the $\mathbf{H}^{-1}$ matrix is modified to become:

$$\left(\mathbf{H}^{\Gamma}\right)^{-1} = \left(\mathbf{A}^{\Gamma}\right)^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}_{05}^{-1} - \left(\mathbf{A}_{22}^{\Gamma}\right)^{-1} \end{bmatrix}$$

Where $\boldsymbol{G}_{05} = \frac{(\boldsymbol{M}-\boldsymbol{P})(\boldsymbol{M}-\boldsymbol{P})^{T}}{\boldsymbol{k}}$, where **M** is the matrix of samples with SNPs encoded as 0, 1, 2 (i.e., the number of reference alleles), **P** is the matrix where each column is filled with the value 1 (i.e., assuming allele frequencies of 0.5 for all loci). The denominator $\boldsymbol{k} = 0.5\boldsymbol{s}$, where $\boldsymbol{s}$ is the total number of SNPs. This corresponds to the genomic relationship matrix proposed by VanRaden (2008) with all allele frequencies assumed to be 0.5. $\mathbf{A}^{\Gamma}$ is pedigree relationship matrix formed with a $\mathbf{\Gamma}$ matrix, and $\mathbf{A}_{22}^{\Gamma}$ is the submatrix of $\mathbf{A}^{\Gamma}$ for the genotyped animals, and $\mathbf{\Gamma}$ is a variance covariance matrix of the MF estimated by $\mathbf{\Gamma} = 8Cov(\mathbf{P})$, as proposed by García-Baccino et al. (2017), where **P** is an $\boldsymbol{m}$ by $\boldsymbol{r}$ matrix of allele frequencies and **r** is the number of MF. Note that this **P** differs from the allele frequency matrix used earlier for individual SNPs in the genomic relationship matrix. Under ssGBLUP without MF, the genomic matrix **G** was constructed using the allele frequencies observed in the genotyped data. Conversely, ssGBLUP that included MF used a fixed allele frequency of 0.5 for all loci. VanRaden (2008) proposed using allele frequencies from base animals, representing an unselected population, to create the genomic matrix. Using an allele frequency of 0.5 in ssGBLUP with MF represents a relationship across individuals in the base pedigree population(s) relative to an unobserved base population with all allele frequencies equal 0.5 (Legarra et al., 2024). The only modification of the **A** matrix to include MF is the assumption that the MF have a self-relationship denoted as $\mathbf{\Gamma}$. The $\mathbf{\Gamma}$ matrix, which models the means within and across founders, was estimated using observed genotypes and pedigree under a generalized least square (**GLS**) approach (Garcia-Baccino et al., 2017) using the gammaf90 software package (Aguilar & Misztal, 2008).

Metafounder classification

This study examined four scenarios to assess the impact of different strategies to build MF for a given data set and pedigree setting:

1) ssGBLUP without MF (NO_MF):

A ssGBLUP that did not include MF nor any UPG was implemented so that all unknown parents in the pedigree are assumed to be unrelated and from a single population, hence having unknown breeding values.

2) ssGBLUP with MF defined by breed (Γ_4):

In this approach, four MF were defined based on the breed of origin, with one MF assigned to HO, one for JE, another for CROSS, and a fourth assigned to the rest of the base animals, assuming their breed of origin was unknown. This approach treated CROSS as a distinct genetic group ("breed") alongside HO, JE, and Unknown. Thus, in the end, the variance-covariance matrix among MF was a 4x4 matrix between the means across SNP and breeds.

3) ssGBLUP with MF defined by breed, sex, and birth year (Γ_{24}):

In this approach, 24 MF were defined based on breed (HO, JE, Unknown), sex, and year of birth (≤2000, 2001–2005, 2006–2010, ≥2011). Here, the CROSS group was modelled within the covariance between HO and JE.

4) ssGBLUP with MF defined by breed, sex, year of birth and crossbreds as a breed (Γ_{32}):

This approach expanded upon $\mathbf{\Gamma_{24}}$ by explicitly treating crossbred animals (CROSS) as a distinct genetic group alongside HO, JE, and Unknown. As a result, metafounders were defined for each combination of breed (HO, JE, CROSS, Unknown), sex, and year of birth, resulting in 32 total metafounders. This distinction allowed animals with mixed ancestry and no known parents to be grouped more consistently, rather than approximating their breed origin via pedigree tracing. In this case, crossbred animals with no parent information were directly assigned to the CROSS metafounder group.

Following Legarra et al (2015), genetic variance parameters obtained from the model with unrelated founders were used to estimate corresponding parameters for the models with MF by scaling it to become;

$$\sigma^2_{related} \approx \frac{\sigma^2_{unrelated}}{1 + \frac{diag(\Gamma)}{2} - \bar{\Gamma}}$$

where the denominator is the scaling factor *k*; $\sigma^2_{unrelated}$ is the variance among unrelated founders. The variance of the breeding values can then be calculated as $\mathrm{var(u)} = \mathbf{H}^{\Gamma}.\,\sigma^2_{\mathrm{related}}$, where $\mathbf{H}^{\Gamma}$ is again the combined relationship matrix described in Legarra et al. (2015).

Comparisons

The four ssGBLUP scenarios were evaluated, where three used different MF classifications and one used a conventional ssGBLUP model without the inclusion of any MF

To confirm these assumptions, we investigated the mean differences in the diagonal and off-diagonals of $\mathbf{A}_{22}$, $\mathbf{G}$, $\mathbf{A}^{\Gamma}_{22}$, and $\mathbf{G}^{\Gamma}$ matrices (defined by MF groups) by correlations and mean differences between these matrices.

Finally, the four sets of ssGBLUP predictions were compared using the validation metrics described above for each studied trait.

Results & Discussion

Elements of matrices

Table 2 shows the summary statistics for the different matrices used in the ssGBLUP computation using APY with a random core size of 30,000. Values of the diagonal and off-diagonal elements of $\mathbf{A}_{22}$ and $\mathbf{G}$ increased in all augmentations of $\mathbf{A}$ and $\mathbf{G}$ that considered $\mathbf{\Gamma}$. The mean, minimum, and maximum values of the diagonal and off-diagonal elements of $\mathbf{A}^{\Gamma 24}_{22}$, $\mathbf{A}^{\Gamma 32}_{22}$, $\mathbf{G}^{\Gamma 24}$, and $\mathbf{G}^{\Gamma 32}$ were similar. This similarity implied that the assignment of an MF to the crossbred base population in $\mathbf{\Gamma}_{32}$ resulted in the little to no effect on the relationship among individuals when compared with modeling the crossbred base population within the covariance between the MF of HO and JE augmented in $\mathbf{\Gamma}_{24}$.

Incorporating MF in $\mathbf{A}_{22}$ increased the correlation between the pedigree and genomic relationship matrices. Correlation between the diagonal elements of A_{22} and $\mathbf{G}$, $\mathbf{A}^{\Gamma 4}_{22}$ and $\mathbf{G}^{\Gamma 4}$ $\mathbf{A}^{\Gamma 24}_{22}$ and $\mathbf{G}^{\Gamma 24}$, $\mathbf{A}^{\Gamma 32}_{22}$ and $\mathbf{G}^{\Gamma 32}$ were 0.18, 0.64, 0.28, and 0.29, respectively. In the same way, the correlation between the off-diagonal elements of A_{22} and $\mathbf{G}$, $\mathbf{A}^{\Gamma 4}_{22}$ and $\mathbf{G}^{\Gamma 4}$ $\mathbf{A}^{\Gamma 24}_{22}$ and $G^{\Gamma 24}$, $\mathbf{A}^{\Gamma 32}_{22}$ and $\mathbf{G}^{\Gamma 32}$ were 0.39, 0.66, 0.46, and 0.47 respectively. In all scenarios, using the $\mathbf{\Gamma}_4$ resulted in higher-than-average diagonals and off-diagonals in the elements of **A** and **G**. These results were expected as including MF has been shown to improve the similarity between the pedigree and genomic relationship matrices compared to the traditional ssGBLUP model (Legarra et al., 2015).

Furthermore, the off-diagonal elements in a pedigree relationship matrix containing MF are expected to be higher than those of a pedigree without MF (Junqueira et al., 2020; Kudinov et al., 2020), as shown in table 2.

Table 2: mean, minimum, and maximum element values of $\mathbf{A}_{22}$, $\mathbf{A}^{\Gamma 4}_{22}$, $\mathbf{A}^{\Gamma 24}_{22}$, $\mathbf{A}^{\Gamma 32}_{22}$, $\mathbf{G}$, $\mathbf{G}^{\Gamma 4}$,$\mathbf{G}^{\Gamma 24}$,$\mathbf{G}^{\Gamma 32}$ from diagonal and off-diagonal[1].

Element	Matrix	Mean	Minimum	Maximum
Diagonal	*$\mathbf{A}_{22}$	1.004	1.000	1.286
	$\mathbf{G}$	1.004	0.779	1.453
	$\mathbf{A}^{\Gamma 4}_{22}$	1.324	1.266	1.551
	$\mathbf{G}^{\Gamma 4}$	1.320	1.121	1.568
	$\mathbf{A}^{\Gamma 24}_{22}$	1.306	1.008	1.504
	$\mathbf{G}^{\Gamma 24}$	1.319	1.120	1.568
	$\mathbf{A}^{\Gamma 32}_{22}$	1.306	1.163	1.504
	$\mathbf{G}^{\Gamma 32}$	1.319	1.120	1.568
Off-diagonal	$\mathbf{A}_{22}$	0.016	0.000	0.666
	$\mathbf{G}$	0.016	-0.216	1.015
	$\mathbf{A}^{\Gamma 4}_{22}$	0.613	0.532	1.154
	$\mathbf{G}^{\Gamma 4}$	0.632	0.397	1.386
	$\mathbf{A}^{\Gamma 24}_{22}$	0.604	0.385	1.073
	$\mathbf{G}^{\Gamma 24}$	0.631	0.395	1.380
	$\mathbf{A}^{\Gamma 32}_{22}$	0.604	0.413	1.073
	$\mathbf{G}^{\Gamma 32}$	0.631	0.396	1.380

*$\mathbf{A}_{22}$ is the pedigree relationship matrix of the genotyped animals; $\mathbf{G}^{\Gamma 4}$, $\mathbf{G}^{\Gamma 24}$, and $\mathbf{G}^{\Gamma 32}$ are the genomic relationship matrices with allele frequencies equal to 0.5 augmented by the Γ_4, Γ_{24}, and Γ_{32}, respectively; $\mathbf{G}$ is the genomic relationship matrix obtained using the VanRaden (2008) method 1; $\mathbf{A}^{\Gamma 4}_{22}$, $\mathbf{A}^{\Gamma 24}_{22}$, $\mathbf{A}^{\Gamma 32}_{22}$, are the pedigree relationship matrices of genotyped animals augmented by $\mathbf{\Gamma}_4$, $\mathbf{\Gamma}_{24}$, $\mathbf{\Gamma}_{32}$ respectively.

Inflation

The slope (b_1) of the regression of adjusted phenotypes on GEBV from reduced datasets measures the dispersion of predictions. A slope close to one indicates no inflation or deflation in GEBV (Mäntysaari et al., 2010). According to Interbull guidelines, b_1 should range from 0.90 to 1.10 for large populations, or be within statistical significance of 1.0 for smaller populations. Table 3 summarizes slopes across traits (DPR, FY, MY, PY, SCS), methods (NO_MF, 4MF, 24MF, 32MF), and groups (CROSS, HO, JE).

In CROSS, NO_MF exhibited severe overdispersion, with slopes well below one for MY (0.52 ± 0.08), PY (0.42 ± 0.09), and FY (0.37 ± 0.09). Introducing 4MF improved dispersion (e.g., MY: 0.63 ± 0.06; PY: 0.51 ± 0.07). However, finer partitions (24MF, 32MF) did not consistently improve slopes and, for MY, slopes declined to 0.45 ± 0.04 (24MF) and 0.54 ± 0.05 (32MF), suggesting potential reintroduction of bias. For low-heritability traits (DPR, SCS), slopes remained far from one and highly variable across scenarios.

In HO and JE, slopes were closer to one across models. HO slopes ranged narrowly (0.68–0.77). For JE, 4MF slightly improved MY slope (0.67 ± 0.03 [NO_MF] → 0.87 ± 0.02 [4MF]), with minimal differences between 4MF, 24MF, and 32MF. These results suggest that coarser MF groupings can reduce overdispersion in CROSS, but finer granularity does not guarantee further improvement and may exacerbate bias.

Overall, slopes were significantly different from 1.0 (*P < 0.05), indicating general inflation in predictions. However, less biased results for evaluations with MF were observed as shown in other studies (e.g., Garcia-Baccino et al., 2017). A potential factor is variance scaling in MF base populations. While Legarra et al. (2015) described theoretical scaling, its practical implementation has been inconsistent (Macedo et al., 2020; Meyer, 2021). Himmelbauer et al. (2024) reported that scaled variances tend to slightly overestimate GEBV. In this study, scaling factors (k) for base animals were 1.002 (4MF), 1.011 (24MF), and 1.015 (32MF), suggesting variance scaling did not contribute to inflation. Breed-specific contributions to the base population, as noted by Kudinov et al. (2022), may explain slope differences across groups.

Suboptimal reference populations and limited crossbred genotypes that did not represent this group in the APY core likely contributed to the overdispersions observed in our study, as shown in Khansefid et al. (2020) and van den Berg et al. (2020).

Table 3: Regression coefficients (b1) and SE of cow-adjusted phenotypes on genomic estimated breeding value from different single-step genomic BLUP (ssGBLUP) scenarios for validation cows.

^{1}Scenario	Group^2	Trait				
		MY	PY	FY	SCS	DPR
NO_MF	HO	0.77	0.55	0.62	0.65	0.20
	JE	0.67	0.6	0.55	0.45	0.19
	CROSS	0.52	0.42	0.37	0.10	0.36
4MF	HO	0.77	0.55	0.70	0.79	0.27
	JE	0.87	0.76	0.69	0.67	0.22
	CROSS	0.63	0.51	0.49	0.29	0.35
24MF	HO	0.68	0.48	0.61	0.78	0.24
	JE	0.89	0.77	0.69	0.65	0.22
	CROSS	0.45	0.37	0.39	0.29	0.28
32MF	HO	0.68	0.49	0.67	0.78	0.24
	JE	0.87	0.76	0.69	0.67	0.22
	CROSS	0.54	0.43	0.44	0.28	0.30

Scenario^1: NO_MF model (single-step genomic BLUP without metafounders); 4MF (single-step genomic BLUP with four metafounders); 24MF (single-step genomic BLUP with 24 metafounders); 32MF (single-step genomic BLUP with 32 metafounders). Group^2 = HO; Holstein (n = 96,295 animal); JE; Jersey (n = 26,436 animals); CROSS (n = 5,099). MF = Metafounder; ^{2}SE: HO ≤0.02 for all traits and scenarios; JE: ≤ 0.06 for all traits and scenarios; CROSS: ≤ 0.18 for all traits and scenarios; MY = milk yield; FY = fat yield; PY = protein yield; SCS = somatic cell score; DPR = daughter pregnancy rate

Predictabilities

Table 4 summarizes predictabilities for MY, PY, FY, SCS, and DPR across models (NO_MF, 4MF, 24MF, 32MF) and groups (CROSS, HO, JE). For MY, HO and JE cows showed moderate, stable predictabilities across all models (HO: 0.41–0.44; JE: 0.40–0.50). In contrast, CROSS animals demonstrated notable gains with MF inclusion, increasing from 0.33 under NO_MF to 0.44 with 4MF, and further to

0.48–0.49 under 24MF and 32MF. Incremental gains beyond 4MF were modest, suggesting diminishing returns with finer metafounder definitions.

For PY and FY, similar trends were observed. JE cows exhibited higher baseline predictabilities (PY: 0.31 NO_MF → 0.37–0.38 MF), while HO showed smaller changes. CROSS animals had the largest improvements, especially under 4MF (e.g., FY: 0.19 NO_MF → 0.26 4MF). Gains under 24MF and 32MF were limited.

For SCS and DPR, purebred predictabilities remained high and stable across all models, while CROSS animals showed improvements from low baselines (e.g., SCS: 0.04 NO_MF → 0.12 4MF → 0.14 24MF/32MF).

These results highlight that MF effects on predictability are trait- and breed-dependent. Coarser MF groupings (4MF) improve CROSS predictions, while finer partitions do not guarantee further accuracy and may introduce unnecessary complexity.

Our findings differ from Cesarani et al. (2023), who reported higher CROSS predictabilities than purebreds using UPG in ssGBLUP. They attributed this to genetic divergence between HO and JE and dense genotype panels (imputed 79K SNPs). In contrast, our study used a 45K SNP panel and a random APY core including only 678 crossbreds (<3%). These factors likely reduced CROSS prediction accuracy despite the inclusion of MF.

A more balanced APY core design, like the breed-stratified approach of Tabet et al. (2025), could better capture genetic variation in small groups like CROSS while maintaining computational efficiency. Combining variance-based core selection with breed stratification may offer a promising strategy for future multi-breed evaluations.

Table 4: Predictive ability (Pearson correlation between genomic estimated breeding values and adjusted phenotype) for the validation cows.

Scenario[1]	Group[2]	Trait				
		MY	PY	FY	SCS	DPR
NO_MF	HO	0.41	0.30	0.33	0.21	0.07
	JE	0.40	0.31	0.26	0.17	0.05
	CROSS	0.33	0.24	0.19	0.04	0.08
4MF	HO	0.44	0.31	0.37	0.25	0.08
	JE	0.50	0.38	0.34	0.22	0.06
	CROSS	0.44	0.33	0.26	0.12	0.10
24MF	HO	0.41	0.28	0.35	0.25	0.08
	JE	0.50	0.37	0.34	0.21	0.06
	CROSS	0.48	0.39	0.29	0.14	0.09
32MF	HO	0.41	0.28	0.36	0.25	0.08
	JE	0.50	0.38	0.34	0.22	0.06
	CROSS	0.49	0.39	0.30	0.14	0.09

Scenario[1]: NO_MF model (single-step genomic BLUP without metafounders); 4MF (single-step genomic BLUP with four metafounders); 24MF (single-step genomic BLUP with 24 metafounders); 32MF (single-step genomic BLUP with 32 metafounders). Group[2] = HO; Holstein (n = 96,295 animal); JE; Jersey (n = 26,436 animals); CROSS; HOxJE animals (n = 5,099). MF = Metafounder; [2]SE: HO ≤0.003 for all traits and scenarios; JE: ≤ 0.005 for all traits and scenarios; CROSS: ≤ 0.013 for all traits and scenarios; MY = milk yield; FY = fat yield; PY = protein yield; SCS = somatic cell score; DPR = daughter pregnancy rate

Stabilities

In HO, stability was high under NO_MF (≥0.87 for all traits) as shown in Table 5, reflecting strong agreement between reduced and complete datasets. Including 4MF slightly reduced stability for production traits such as PY (0.87 → 0.77) and MY (0.87 → 0.80), while traits with low heritability (SCS, DPR) remained highly stable (≥0.93). Increasing MF resolution to 24MF and 32MF had negligible additional effects, with correlations for MY and

PY ranging from 0.76 to 0.80 and SCS/DPR remaining ≥0.93. These findings suggest that, for HO, finer MF groupings increased model complexity without enhancing stability and may have even slightly destabilized predictions for certain traits.

In JE, stability was similarly high across all traits in NO_MF (e.g., MY and FY = 0.93) and remained largely unchanged with MF inclusion. Minor improvements in MY stability (0.93 → 0.94 under 4MF) were observed, but finer MF resolutions (24MF, 32MF) did not yield further gains, indicating limited impact of MF on stability in this breed.

In contrast, CROSS animals showed lower stability under NO_MF (e.g., MY = 0.59, PY = 0.52, FY = 0.69) compared to purebreds. MF inclusion modestly improved stability (e.g., MY: 0.59 → 0.61 under 4MF), with larger gains observed under 24MF (MY: 0.73) and 32MF (MY: 0.74). Similar trends were noted for other traits, suggesting that finer MF groupings may better account for heterogeneity in crossbred populations.

These results highlight potential trade-offs. In purebreds, finer MF schemes increased model complexity without clear benefits and may have introduced overparameterization relative to the data. In CROSS, finer MF improved stability but did not consistently translate to higher predictive ability or slopes closer to one. This decoupling suggests that stability alone cannot fully evaluate model performance and must be interpreted alongside other validation metrics and trait architecture.

As Legarra and Reverter (2018) emphasized, high stability does not necessarily reflect improved accuracy. For traits like MY and PY in purebreds, high stability may partly reflect that most genetic variance was captured by earlier data, limiting the impact of new phenotypes. Conversely, in traits with lower heritability (e.g., DPR, SCS), MF inclusion improved stability, indicating that such traits may benefit more from additional information introduced by metafounders.

Stability should therefore be interpreted cautiously. While desirable for routine evaluations, it primarily measures agreement between evaluations and does not indicate which evaluation is more accurate. For traits with low h^2, high stability may reflect unresponsiveness to new data, which could limit genetic progress.

Table 5: stability (correlation between genomic estimated breeding values estimated in the complete and reduced datasets) for validation cows.

Scenario[1]	Group[2]	Trait				
		MY	PY	FY	SCS	DPR
NO_MF	HO	0.87	0.87	0.89	0.95	0.91
	JE	0.93	0.92	0.93	0.92	0.89
	CROSS	0.59	0.52	0.69	0.79	0.88
4MF	HO	0.80	0.77	0.83	0.94	0.93
	JE	0.94	0.92	0.92	0.92	0.92
	CROSS	0.61	0.50	0.54	0.76	0.88
24MF	HO	0.76	0.72	0.78	0.93	0.93
	JE	0.92	0.9	0.91	0.92	0.94
	CROSS	0.73	0.65	0.62	0.82	0.91
32MF	HO	0.80	0.73	0.80	0.93	0.93
	JE	0.92	0.90	0.91	0.92	0.93
	CROSS	0.74	0.65	0.64	0.83	0.91

Scenario[1]: NO_MF model (single-step genomic BLUP without metafounders); 4MF (single-step genomic BLUP with four metafounders); 24MF (single-step genomic BLUP with 24 metafounders); 32MF (single-step genomic BLUP with 32 metafounders). Group[2] = HO (n = 96,295 animal); JE (n = 26,436 animals); CROSS (n = 5,099). MF = Metafounder; [2]SE: HO ≤0.001 for all traits and scenarios; JE: ≤ 0.001 for all traits and scenarios; CROSS: ≤ 0.011 for all traits and scenarios; MY = milk yield; FY = fat yield; PY = protein yield; SCS = somatic cell score; DPR = daughter pregnancy rate

Conclusions

This study demonstrated that incorporating metafounders (MF) into genomic evaluation models for Holstein and Jersey cattle, as well as their crossbreds, can result in differences in prediction metrics, with the effects varying by trait, breed, and metafounder configuration. While certain MF classifications (eg, 4MF) reduced bias and improved regression slopes in crossbreds for some traits, others had minimal effects, especially for purebred Holstein. However, the added model complexity slightly reduced stability for traits with higher heritability, such as milk yield and protein yield. Overall, while MF provides a promising approach to address pedigree missingness in multibreed evaluations, its application should be tailored to the trait heritability and population composition to avoid potential overfitting and ensure accurate genetic predictions.

Acknowledgments

The authors acknowledge Zoetis Genetics for providing the data for this study. The research described here was a part of the corresponding author's internship program sponsored through the research agreement between the University of Connecticut (Storrs) and Zoetis. The authors have not stated any conflicts of interest.

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Single-step genetic evaluation for claw health traits in Switzerland

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Abstract

In Switzerland, a resource project was launched in 2019 to improve claw health in Swiss cattle. This project marked the beginning for the development of the first genetic evaluation for claw health traits in Swiss dairy cattle. Data recorded by claw trimmers during routine care was used to develop a single-step genetic evaluation for the most common dairy cattle breeds Holstein, Swiss Fleckvieh, Simmental, Brown Swiss and Original Braunvieh.

A key advantage of this dataset is its comprehensive inclusion of all healthy cows observed during routine care. From 2019 to 2024, a total of 104,276 records were collected for the multi-breed evaluation of Holstein cattle (encompassing Holstein, Swiss Fleckvieh, and Simmental), while 33,464 records were documented for Brown Swiss (Brown Swiss and Original Braunvieh). Breeding values were predicted for four distinct traits: dermatitis digitalis (DD), white-line disease (WL), other infectious claw diseases (INF), and other non-infectious claw diseases (NINF). The prevalence rates of DD, WL, INF, and NINF were observed as 20.9%, 9.9%, 45.8%, and 20.7% respectively in the Holstein evaluation, while in the Brown Swiss evaluation, they were found as 7.8%, 10.2%, 37.6%, and 13.5%.

A multi-trait animal model with binary coding of the trait was fit in the ssGTaBLUP evaluation, with heritability estimates ranging from 3% to 9% determined for the four traits across both populations. A top-bottom comparison revealed that daughter prevalence among sires with the highest and lowest estimated breeding values (EBV) varied by 14% to 35%. An index incorporating breed-specific weights for the four traits has been developed and is now published for all Swiss breeders.

A primary challenge in developing the genetic evaluation was the scarcity of phenotypic data alongside a substantial population of genotyped animals that exhibited limited genetic correlation with individuals supplying phenotypic records.

Key words: Claw health, single-step genetic evaluation, dairy cattle, multi breed evaluation

Introduction

Claw health represents the third leading cause of culling in Swiss dairy cattle, following mastitis and fertility challenges. Claw diseases and the resultant lameness significantly affect animal health and welfare as well as herd productivity, primarily due to treatment costs and decreased milk yield. Previous research has estimated that associated economic losses can range from several hundred to over one thousand dollars per case and animal (Dolecheck and Bewley, 2018).

A resource project was initiated in 2019 to enhance claw health in Swiss cattle. The main objective was to implement systematic recording of claw health data by hoof trimmers during routine care. Hoof trimmers received training to ensure standardized and consistent documentation of claw diseases. In addition to advancing management practices, a key objective of the project and this study was to establish the first Swiss genetic evaluation for claw health traits and to improve dairy cattle's claw health. Utilizing the comprehensive genomic data available for dairy cattle, a single-step evaluation was developed to maximize the utility of this information. The resulting breeding values will support breeders in sustainably improving the claw health of Swiss dairy cattle through breeding.

Materials and Methods

Data

Data recorded by claw trimmers during routine care was collected through the resource project 'Gesunde Klauen' (https://gesundeklauen.unibe.ch, access date 2025/07/30) for multiple cattle breeds. The raw data set included 286,138 records of both diseased and healthy cases from 2019 to 2024. Thus, having all sound and diseased records solved the question of reconstituting the contemporary groups. Disease recording was done according to ICAR definitions (ICAR, 2020). Pedigree and genotype data were obtained from the three Swiss cattle breeding organizations: Braunvieh Schweiz, swissherdbook, and Holstein Switzerland.

During quality control, records lacking identity information, herd information or disease codes were excluded. Data pertaining to the principal dairy cattle breeds in Switzerland – Holstein, Swiss Fleckvieh, Simmental, Brown Swiss, and Original Braunvieh – were retained. Typically, animals underwent trimming twice annually. Phenotypic data were compiled as records per month and animal. For Holstein multi-breed evaluation, 104,276 records were analysed (including Holstein, Swiss Fleckvieh, and Simmental). 33,464 records were used for Brown Swiss evaluation (Brown Swiss and Original Braunvieh).

Genotype data obtained through standard imputation included 114,640 SNPs for 490,761 animals from Holstein dataset and 146,609 animals corresponding to Brown Swiss evaluation. The Holstein dataset contained 5,284 cows with both phenotype and genotype records, while the Brown Swiss dataset comprised 2,894 cows with these records.

Trait definition

Due to low prevalence of certain diseases, genetic evaluation was limited to dermatitis digitalis (DD) and white-line disease (WL) as individual traits. Remaining diseases were combined into two groups: other infectious diseases (INF) and non-infectious diseases (NINF). INF comprised heel horn erosions, interdigital dermatitis, and interdigital phlegmon. NINF included interdigital hyperplasia (limax), asymmetric, corkscrew, and scissor claws, concave dorsal wall, double sole, axial, horizontal, and vertical horn fissure, thin sole, sole hemorrhage, sole bulb and toe ulcer, and toe necrosis.

Genetic model

A linear multi-trait repeated animal model was implemented to estimate variance components and genomic breeding values. Fixed effects included parity, trimmer by year, stage of lactation, year-month. Recombination and heterosis were also incorporated as fixed effects into the Holstein multi-breed evaluation. The random effects comprised herd-year-season, permanent environment, and animal genetic effect. Traits were coded as binary variables (0 or 1).

Variance components were estimated with the VCE software (version 6.0.2; Neumaier and Groeneveld, 1998) and the four-trait animal model, applied separately to the Holstein and Brown Swiss datasets.

Genetic evaluation

Single-step genomic breeding values were predicted using the ssGTaBLUP model (Mäntysaari et al., 2017) implemented in the MiX99 software package (Strandén and Lidauer, 1999). Reliability estimates for these breeding values were assessed with snp_blup_rel (Zaabza et al., 2020), executed within the MiX99 environment. Estimated breeding values (EBV) were standardized to a mean of 100 and a standard deviation of 12 for publication.

Validation of genetic evaluation

A top-bottom comparison was performed to validate the genetic evaluation process. The average prevalence among daughters was calculated for sires with high reliability (Brown Swiss ≥ 0.35, Holstein ≥ 0.6). These averages were then compared between the sires within the top 10% and bottom 10% of EBV.

Results & Discussion

Prevalence rates for the traits DD, WL, INF, and NINF were observed at 20.9%, 9.9%, 45.8%, and 20.7% respectively in the Holstein evaluation, and at 7.8%, 10.2%, 37.6%, and 13.5% in the Brown Swiss evaluation. The prevalences of DD, WL, and NINF align with findings from Holstein and Brown Swiss populations in other countries (Johansson et al., 2011; Köck et al., 2019; Malchiodi et al., 2018). The comparatively higher prevalence of INF may be attributed to trimmers being instructed to record heel horn erosion with high sensitivity.

Heritability estimates for the four evaluated traits and two assessments of Holstein and Brown Swiss ranged from 3% to 9%, as detailed in Table 1. The highest estimate was recorded for DD in the Holstein evaluation, while the lowest was noted for DD in Brown Swiss. These findings are consistent with previously reported heritability values from studies conducted in other countries (Chapinal et al., 2013; Charfeddine et al., 2018). The highest heritability estimate for DD in Holstein is expected, owing to the more extensive dataset and greater prevalence observed in the Holstein evaluation compared to the Brown Swiss assessment. Additionally, the trait is specifically characterized as a single disease rather than a group trait, enhancing the precision of the evaluation.

Table 1: Heritability estimates and standard errors for claw health traits in the two evaluations

Trait	Holstein	Brown Swiss
Dermatitis digitalis	0.09 (0.003)	0.03 (0.006)
White-line disease	0.05 (0.004)	0.07 (0.009)
Other infectious diseases	0.04 (0.002)	0.04 (0.004)
Other non-infectious diseases	0.06 (0.004)	0.04 (0.007)

The EBV of genotyped sires for the four assessed traits ranged from 53 to 139 in Holstein and from 68 to 123 in Brown Swiss, following standardization. Among Holsteins, the mean reliabilities for DD were 0.84 for proven bulls with a minimum of 20 phenotyped daughters in 10 herds, and 0.43 for selection candidates (Figure 1). In the Brown Swiss evaluation, these averages were 0.67 and 0.24, respectively (Figure 2). The reliability estimates were comparable across all four traits. Generally, the reliabilities observed in Holstein evaluations exceeded those for Brown Swiss, a difference attributable to the greater number of phenotypic records and genotyped animals available for Holstein analyses.

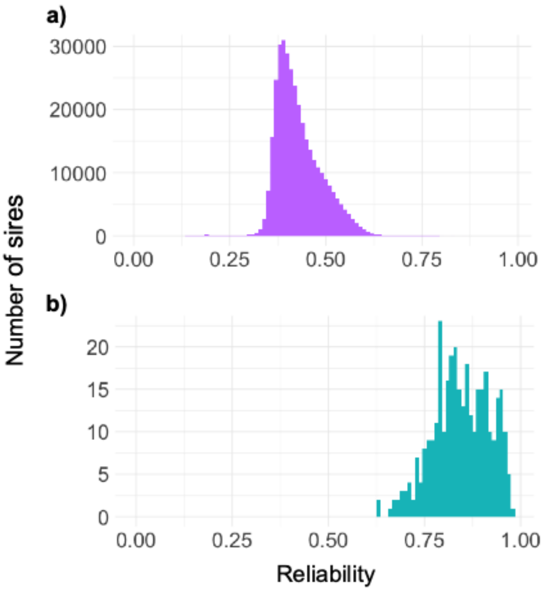

Figure 1. Reliability estimates for dermatitis digitalis (DD) in Holstein for selection candidates (a) and proven bulls (b).

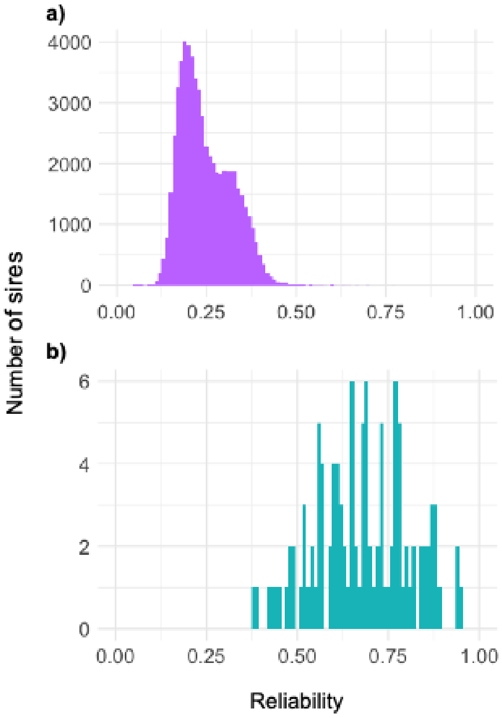

Figure 2. Reliability estimates for dermatitis digitalis (DD) in Brown Swiss for selection candidates (a) and proven bulls (b).

Validation of the EBV through top-bottom comparison revealed differences in daughter prevalence ranging from 14% to 35% across various traits and evaluations. These results are influenced by the average prevalence of the specific disease or disease group under consideration. For example, the top-bottom comparison for DD in Holstein indicated a 32% difference in prevalence between top and bottom sires (Figure 3), while in Brown Swiss cattle, the same trait demonstrated a 14% difference (Figure 4). Both figures illustrate a distinct separation between the two groups, providing strong evidence for the validity of the genetic evaluation for claw health traits. These findings confirm that selecting sires with higher EBV will contribute to genetic improvement in claw health traits.

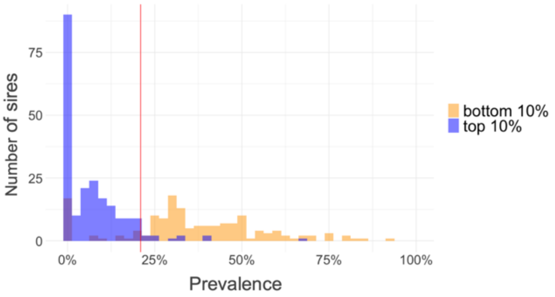

Figure 3. Top-bottom comparison for dermatitis digitalis (DD) in Holstein. The red line indicates the population mean prevalence.

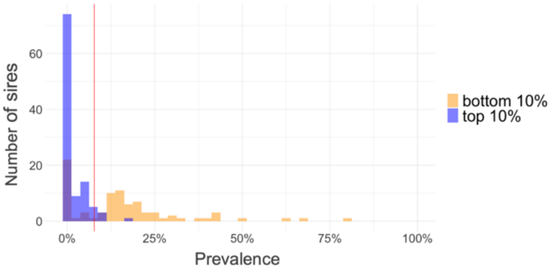

Figure 4. Top-bottom comparison for dermatitis digitalis (DD) in Brown Swiss. The red line indicates the population mean prevalence.

In pursuit of enhanced overall claw health, the EBV of the four traits were consolidated into an index. This EBV index is intended to streamline farmers' selection process during mating decisions. The index weights for these traits were determined specifically for each evaluation and established through consultation with veterinarians involved in the resource project. Weightings were calculated based on both the prevalence of each trait within the respective evaluation and their relative economic significance.

For the Brown Swiss evaluation, all four traits were assigned equal weight in the final claw health index. In contrast, within the Holstein evaluation, DD received a weight of 0.5, WL and INF each received 0.125, and NINF was weighted at 0.25. A modest positive genetic trend is evident for the Brown Swiss breed (Figure 5), with approximately 8 index points gained over the past 20 years – representing three-quarters of a standard deviation. For the Holstein breed, the trend is more pronounced, with a gain of 13 index points or roughly one standard deviation over the same period (Figure 6). Despite the absence of a dedicated selection tool during this time frame, farmers recognized the importance of claw health and made intuitive decisions accordingly. While indirect selection through other traits is theoretically possible, it is considered unlikely. No high genetic correlations with previously selected traits could be identified.

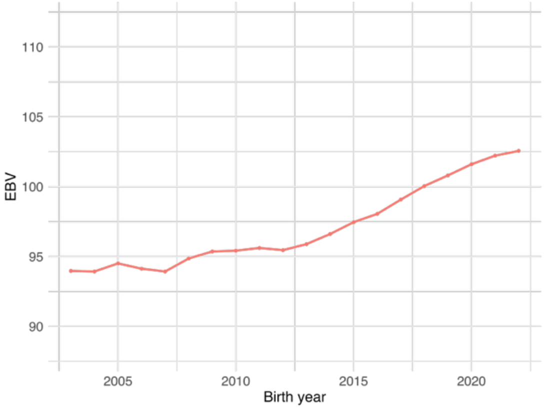

Figure 5. Genetic trend in the claw health index of Brown Swiss for individuals born from 2003 to 2022.

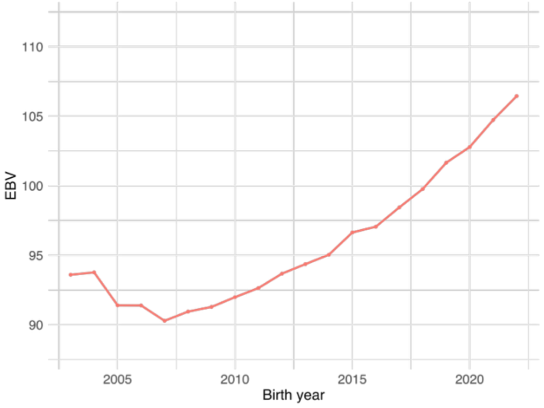

Figure 6. Genetic trend in the claw health index of Holstein for individuals born from 2003 to 2022.

By providing specific EBV for claw health traits, this positive trend can be sustained. Achieving the long-term objectives of the resource project and study is possible through careful consideration of the proposed index and the potential future integration of EBV into the overall selection index for Swiss dairy cattle breeds.

The primary challenge in developing the genetic evaluation was the limited availability of phenotypic data. Despite having five years of recorded information, the database remained constrained due to the small number of participating hoof trimmers, the relatively low population of animals in Switzerland, and their distribution across various breeds. The genetic evaluation was made possible by employing the single-step method and leveraging genomic information. Nevertheless, the abundance of genotypes presented an additional challenge, as most genotyped animals showed weak genetic and genomic connections to those with available phenotypic records. The majority of genotypes were sourced from international bulls through genotype exchange programs.

Conclusions

The first single-step genomic EBV for claw health traits in Switzerland have been predicted. Heritability estimates for four distinct traits ranged from 3% to 9%, based on data from the five major Swiss dairy cattle breeds: Holstein, Swiss Fleckvieh, Simmental, Brown Swiss, and Original Braunvieh. These EBV were used to develop a claw health index, considering evaluation-specific weights to provide Swiss dairy breeders with a straightforward selection tool. Given the observed positive genetic trend, there is potential to further strengthen the genetic improvement of claw health in the future. The next phase involves participating in the development of the new MACE EBV for claw health traits. Following successful validation, we intend to integrate the MACE EBV into our single-step pipeline, leveraging international data to further enhance our genetic evaluation.

Acknowledgments

We gratefully acknowledge the ‘Gesunde Klauen’ project and its funders, in particular the Swiss Federal Office for Agriculture, for supplying the claw health data. We also extend our appreciation to the breeding organizations Braunvieh Schweiz, swissherdbook, and

Holstein Switzerland for providing pedigree and genotype data.

The genetic evaluation team of Qualitas is thanked for the support and exchanges during the work.

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An optimized single-step SNP BLUP model for calf fitness in German Holstein

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Abstract

A single-step SNP BLUP was introduced in routine official evaluation of German Holstein in April 2025 for all traits, including an early-measured trait, calf fitness, defined as calf survival between day 3 and 15 months after birth. Prior to the single-step model implementation, a mixed reference population of bulls and calves was set up for the calf fitness genomic evaluation using a multi-step genomic model. During the testing phase of the single-step model, an unrealistic, strong genetic trend of calf fitness was observed in genotyped animals, when compared to the multi-step genomic model or pedigree-based conventional model. Having searched for plausible causes for the overestimation, we detected a much lower mortality rate for genotyped calves than non-genotyped ones, particularly for the early periods from day 3 to 120 after birth. Although all female calves were genotyped under the whole-herd genotyping scheme in Germany, farmers did not always take genotyping sample right after the birth of a calf, causing a delay in genotyping for the early periods of the trait calf fitness. In addition, there were limited economic incentives for farmers to genotype dead calves. To solve the overprediction bias of the calf fitness evaluation, we developed a new single-step model by using only genotypes of sires of all female calves with phenotypic data. Genomic breeding values of the genotyped calves and all other genotyped animals were indirectly predicted based on SNP effect estimates and residual polygenic effect estimates of all the genotyped sires from the new single-step model. Genomic validation showed a slightly higher accuracy of the new single-step model using sire genotypes than the original model using genotypes of all animals. In comparison to a significant over-prediction for the original model, the new single-step model using only the sire genotypic records gave an almost unbiased genomic prediction. Genetic trends in genotyped AI bulls or female animals were no longer overestimated with the new single-step model. The problem of inflated genomic prediction of the original single-step model seems to be solved by using only the genotypic data of sires of female calves.

Key words: single-step model, calf fitness, genomic evaluation, prediction bias

Introduction

Calf fitness (CF) is an economically important trait for dairy farmers which was defined as female dairy calf survival from day 3 to 15 months / 458 days after birth for German dairy cattle breeds. The whole-time span was divided into five periods: days 3 to 14, 15 to 60, 61 to 120, 121 to 200, and 201 to 458, that were treated as genetically correlated traits with a multi-trait linear animal model (Heise et al. 2016).

Prior to the official implementation of a single-step SNP BLUP genomic model (SSM, Liu et al. 2014) for all evaluated traits in German Holstein in April 2025, genotype records of all animals born from 2005 onwards were used also for trait CF in the testing period of the model SSM. Thanks to the whole-herd female calf genotyping scheme introduced in 2019, more than 1 million German Holstein female calves with phenotypic CF records had also genotype data available for genomic evaluations. An unexpected overestimation of genetic trend in the genotyped animals was, however, identified for trait CF during the test phase of the SSM model. To solve the problem of overestimated genetic trend in trait CF, an alternative single-step model needed to be developed.

The objectives of this study were 1) to identify causes of the inflated genomic prediction of the single-step model using all genotype data; 2) to develop a new single-step model for removing the overestimation bias; and 3) to conduct genomic validation for the two single-step models with a full and a truncated data set.

Materials and Methods

Phenotypic, genotypic and pedigree data from August 2024 (2408) were obtained for the investigation on the trait CF. Following the Interbull GEBV test rules (Mäntysaari et al. 2010), four years of phenotype data were deleted to simulate a genomic valuation in August 2020 (2008t). Two SSM models were compared: using genotype data of all animals including all female calves and using only genotype data of sires of female calves with phenotypic records. Table 1 describes the genotype and phenotype data for the full evaluation 2408 and the truncated evaluation 2008t.

Table 1. Phenotype and genotype data for the full (2408) and truncated evaluation (2008t)

Data set	Female calves with phenotypes	Genotyped calves	Genotyped sires
2408	13,273,996	1,075,268	36,325
2008t	10,733,873	501,653	26,578
Ratio	81%	47%	73%

The total number of genotyped Holstein animals in both evaluations was 1,631,843, including 1,433,599 females and 198,244 male animals. All the genotyped animals were born in 2005 and later due to the left truncation of genotype data (Alkhoder et al. 2024). Figure 1 shows the numbers of female dairy calves with trait CF and genotyped Holstein female calves with trait CF. The solid lines represent the numbers of female dairy calves in the full evaluation 2408 with phenotypic CF records (in blue) and genotyped Holstein female calves (in orange). The dotted lines denote the numbers of female dairy calves with phenotypes in the truncated evaluation 2008t (in black) and with both phenotype and genotype data (in orange).

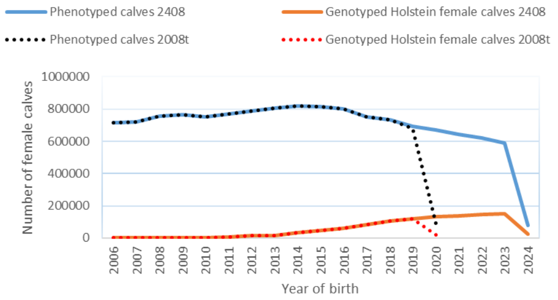

Figure 1. Numbers of female calves in the full evaluation 2408 and truncated evaluation 2008t

Figure 2 shows the number of genotyped sires of the female calves with phenotypic CF in the full evaluation 2408 (solid line) and in the truncated evaluation 2008t (dotted line).

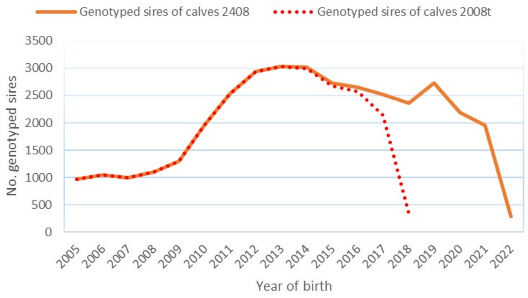

Figure 2. Numbers of genotyped Holstein sires of female calves in the full evaluation 2408 and truncated evaluation 2008t

Mortality rate of dairy female calves

Germany has run a whole-herd genotyping scheme in participating herds since 2016, where all newborn dairy female calves are to be genotyped. For legitimate reasons, farmers do not always take genotyping samples immediately after birth of a calf, causing a delay in genotyping. Furthermore, there is limited incentive for farmers to genotype dead or even sick calves. Based on all 615,927 Holstein female calves born in 2022 which had opportunity to reach the end of CF trait definition (458 days) in the evaluation of April 2025. Figure 3 shows the mortality rates of 148,427 genotyped and 467,500 non-genotyped Holstein calves with respect to the five periods of trait CF. For the first period of CF, non-genotyped calves have a mortality rate of 2.46% that is six times higher than that of genotyped calves, 0.41%. Similarly, the non-genotyped calves are 3 times more likely to die than the genotyped ones in the second period.

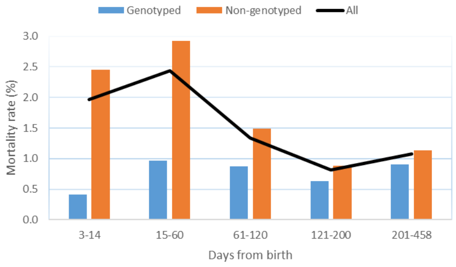

Figure 3. Mortality rates of genotyped and non-genotyped Holstein female calves

A multi-step SNP BLUP genomic model

Under the multi-step genomic model (MSM) for all other evaluated traits in German Holstein (Liu et al. 2011), a SNP BLUP model was applied to deregressed EBV of reference bulls and calves for trait CF. In the full evaluation 2408 there were 1,055,144 reference calves and 13,077 reference bulls representing their non-genotyped calves.

Two single-step SNP BLUP genomic models

For trait CF, we compared two SSM models: one using all genotype records including all calves and the other using only genotype data of sires of the female calves. A single-step SNP BLUP model (Liu et al. 2014) was applied to the phenotype data and the two genotype data sets. For the SSM with sire genotypes, GEBV of all the other genotyped animals were indirectly predicted, following the weekly genomic evaluation procedure (Alkhoder et al. 2024a).

Genotyped Holstein AI bulls and female calves

For trait CF two main groups of genotyped animals were chosen to investigate the impact of the two SSM models: genotyped Holstein AI bulls and genotyped Holstein female calves. Both animal groups were highly relevant for breeding and most affected by the SSM model change as well. Figure 4 shows the number of AI bulls by year of birth with a total of 8,391 genotyped Holstein AI bulls owned by German AI studs born from 2005 to 2023. Numbers of the genotyped Holstein female calves having trait CF born in 2010 and later are shown in Figure 5. The total number of the genotyped Holstein calves is 1,072,492 in the evaluation 2408.

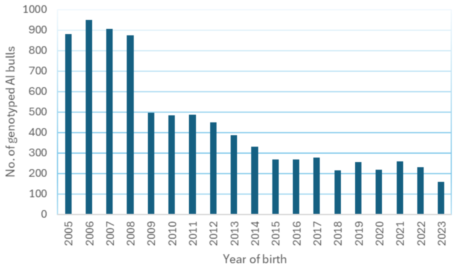

Figure 4. Number of genotyped Holstein AI bulls owned by German AI studs

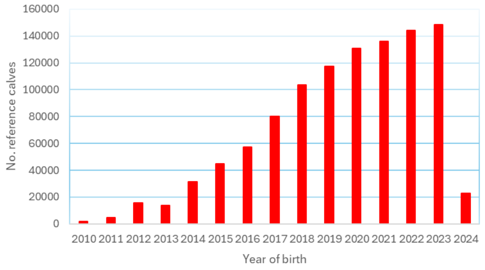

Figure 5. Number of genotyped Holstein female calves with phenotypic records

Genomic validation via GEBV Test

Following Interbull GEBV Test rules, a total of 980 validation bulls were defined based on the full and truncated data sets. The most recent version of the GEBV Test software from September 2024 was used at the time of conducting the genomic validation. To investigate the impact of dependent variable on the validation results, both deregressed GEBV and GEBV were used as target variable. For the MSM model, an earlier genomic validation with a linear regression model (Legarra and Reverter, 2018) was performed using data from a full evaluation in April 2023 (2304) and from a truncated evaluation in April 2021 (2104). For this special validation, the original SSM with genotype data of all animals was evaluated besides the MSM model. Furthermore, GEBV of 355 validation bulls from the full evaluation 2304 were regressed on GEBV from the truncated evaluation 2104 for the regression analysis.

Results & Discussion

The single-step genomic full evaluation, 2408, was run using the two genotype data sets of German Holstine for trait CF: using genotypes of all animals and using only genotypes of sires of the female calves with own phenotypic records in CF. For the GEBV test, the single-step evaluation based on the truncated phenotypic data, 2008t, was conducted for the two genotype data sets as well. In addition, we further ran the MSM model using the full data set 2408 and truncated 2008t. All the SSM evaluations were run with software MiX99 (Strandén and Lidauer, 1999), whereas our own programs were used for the MSM evaluations.

Earlier genomic validation results

Table 2 shows results of the earlier genomic validation using the linear regression method (Legarra and Reverter, 2018) by comparing the full evaluation 2304 to truncated evaluation 2104. It can be seen for both SSM and MSM models that the model R^2 value is relatively high and b_1 value close to 1. However, caution needs to be taken when interpreting the validation results, because the validation bulls have low reliability values in comparison to other traits, between 0.5 and 0.6 in the full evaluation 2304, for the low heritability trait CF. The high R^2 values may also be attributed to the lower contribution of own calves' phenotypic data to the total reliability of the validation bulls. Using GEBV as dependent variable for the linear regression may partially lead to the b_1 values close to 1, too.

Table 2. Genomic validation results using data from the full evaluation 2304 and truncated evaluation 2104

Genomic model	Number of validation bulls	R^2 value	b_1 value
Single-step	355	0.61	1.04
Multi-step	355	0.40	0.92

Genomic validation results of the two single-step models

Tables 3 and 4 give results of genomic validation for both SSM models via Interbull GEBV Test software using data from the full evaluation 2408 and truncated evaluation 2008t. The total number of validation bulls was 980. The two SSM models show significantly lower R^2 values than those in Table 2, indicating that the dependent variable deregressed GEBV results in a lower R^2 value than the dependent variable GEBV. Another explanation for the lower R^2 values is the number of years in the data truncation, 4 years for the validation in Table 3 versus 2 years for the validation in Table 2. Based on the regression slope b_1 values, we can conclude that the SSM model using genotype data of all animals failed the GEBV test, leading to overestimated candidate GEBV.

Table 3. Genomic validation results of the two single-step models using data from the full evaluation 2408 and truncated evaluation 2008t

Deregressed GEBV as dependent variable	R^2 value	b_1 value	Pass
Using only sire genotypes	0.191	0.954	PASS
Using all genotypes	0.164	0.849	FAIL

As an alternative form of dependent variable in the GEBV Test, GEBV of validation bulls from the full evaluation 2408 were regressed on those of the truncated evaluation 2008t. Table 4 shows genomic validation results of the two SSM models with GEBV as dependent variable. The R^2 values of both SSM models are nearly equal and higher than the validation using deregressed GEBV in Table 3. Based on the regression slope b_1 estimates, the two SSM models pass the GEBV test. However, the b_1 value of the SSM using all genotype data, 0.933, deviates more from its expected value of 1.

Table 4. Genomic validation results of the two single-step models using GEBV as dependent variable for the regression analysis

GEBV as dependent variable	R^2 value	b_1 value	Pass
Using only sire genotypes	0.444	0.963	PASS
Using all genotypes	0.436	0.933	PASS

GEBV of the genotyped Holstein AI bulls

Figure 6 shows genetic trends of GEBV in the genotyped Holstein bulls born between 2005 and 2023. GEBV of the AI bulls are expressed in genetic standard deviations (σ_g) in Figure 6.

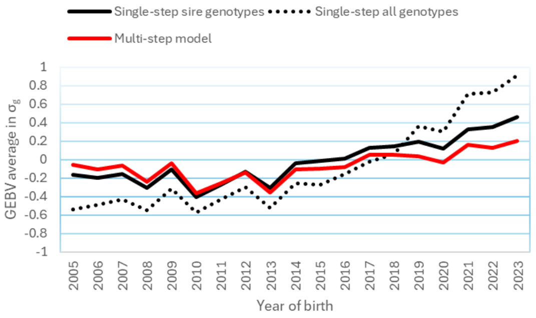

Figure 6. Genetic trends of the three genomic models in the genotyped Holstein AI bulls

The SSM model using all genotype data (dotted black line) which failed the GEBV Test (see Table 3) has too high genetic trend, with a genetic progress of 1.4 genetic standard deviations in las 10 years between 2013 and 2023, despite the fact no direct selection has been imposed on this trait CF in German Holstein. The new, optimized SSM model using only sire genotype data (solid black line) has brought down the genetic trend significantly, to a level much closer to the MSM model. For information, the genetic trend of the MSM model is equal to conventional evaluation for the genotyped AI bulls with calf phenotype data.

GEBV variances of the AI bulls by birth year are given in Figure 7. All the three genomic models have nearly equal GEBV standard deviations within the birth years.

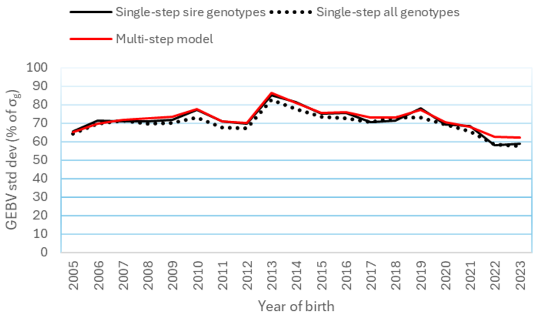

Figure 7. GEBV standard deviations of the three genomic models for the genotyped Holstein AI bulls

Figure 8 shows GEBV correlations between all pairs of three 3 genomic models. The new SSM model with only sire genotype has high GEBV correlations with either the SSM model using all genotype data (solid black line) or the MSM model (dotted green line). The MSM model and the SSM with all genotypes (dashed blue line) have the lowest GEBV correlations.

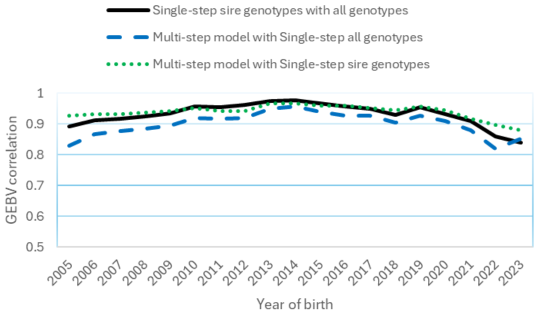

Figure 8. GEBV correlations between the genomic models for the genotyped Holstein AI bulls

GEBV of the genotyped Holstein female animals

Regarding the genetic trends of the 3 genomic models in the genotyped Holstein female calves, we can see in Figure 9 that the SSM using all genotype data (dotted black line) has severely overestimated GEBV of the female calves, due to the much lower mortality rate of genotyped than non-genotyped calves (see Figure 3). However, the unrealistically high genetic trend is reduced significantly for the SSM model when only the genotype data of sires were used (solid black line). The GEBV averages by birth year of the new SSM with only sire genotypes are now only slightly higher than those of the MSM model (solid red line).

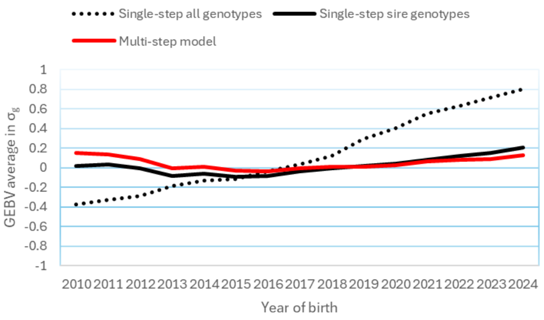

Figure 9. Genetic trends of the three genomic models in the genotyped Holstein female animals

In addition, GEBV variances in the genotyped female calves are compared among the genomic models (Figure 10). Despite the large trend difference in genotyped animals between the two SSM models, the genotyped Holstein female calves have nearly equal GEBV variances (dotted and solid black lines), probably due to the rather low heritability of trait CF. The MSM model has lower GEBV variance (solid red line) than the two SSM models, which may be explained by the contribution of non-genotyped relatives with phenotype data to the female calves GEBV of the SSM model.

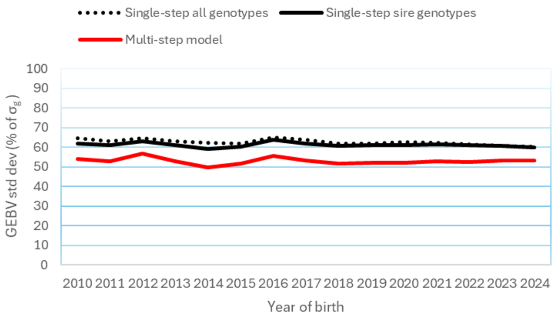

Figure 10. GEBV standard deviations of the genomic models in genotyped Holstein female calves

GEBV correlations between the genomic models are shown in Figure 11 for the genotyped Holstein female calves. The highest GEBV correlations are found between the MSM model and SSM with only sire genotypes (dotted green line), whereas the GEBV correlations between the MSM model and the SSM using all genotypes are lowest (dashed blue line).

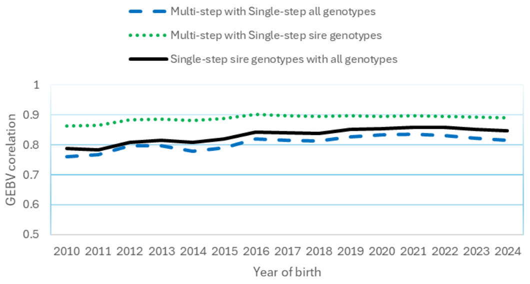

Figure 11. GEBV correlations between the genomic models for the genotyped Holstein female calves

The MSM model with a mixed reference population of bulls and calves did not show the problem of overestimated GEBV for the early measured trait CF, partly due to the reference bulls whose EBV containing phenotype data of both live and dead calves. As another contributing factor, the pseudo-phenotype data of reference bulls or calves in the MSM genomic evaluation were deregressed conventional EBV of bulls and calves, which had been estimated in the preceding conventional evaluation without consideration of any genotype data. Thus the problem of genotyped calves having a much lower mortality rate than the non-genotyped calves could not have any impact on the conventional EBV at the preceding step and on the subsequently generated deregressed conventional EBV of the reference bulls or calves.

Conclusions

The single-step genomic model using genotype data of all animals appears to give biased genomic prediction for the early-measured trait calf fitness, when genotyping of some calves are delayed with respect to the trait definition or there is a limited genotyping of dead calves. The inflated genomic prediction, observed in the single-step evaluation using all genotype data for calf fitness in German Holstein, occurred even under the whole-herd female genotyping scheme in Germany, where all female calves are systematically genotyped.

A strategy for solving the problem of inflated genomic prediction was developed by using only genotype data of sires of phenotyped female calves, because the sires have both dead and live calves and almost all the phenotyped calves have a genotyped sire. Following Interbull GEBV test rules, phenotypic data in the last four years were removed from the full evaluation to perform a genomic validation. The new, optimized single-step model resulted in a slightly higher accuracy and a nearly unbiased regression slope estimate than the original single-step model. For the low heritability trait calf fitness where validation bulls have a relatively low reliability, we found that the deregressed GEBV are clearly more appropriate as dependent variable of the linear regression than the GEBV of validation bulls. In case of a high number of reference animals for the reduced genomic evaluation, 4-year data truncation is preferred to a 2-year data cut to achieve more realistic validation results.

By comparing the new single-step model to the previous one using genotype data of all animals, we found that the genetic trends in AI bulls and genotyped female calves were reduced significantly and GEBV become more accurate, with slight change in GEBV variances. Finally, we can draw a conclusion that the inflated single-step prediction problem of the early-measured trait calf fitness has been solved by using only genotype data of calf sires.

Acknowledgments

Colleagues of Nordic Genetic Evaluation Center (NAV) are acknowledged for a video meeting on 26 September 2024 on young stock survival evaluation.

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Alternative Approaches to handling of missing parents in genetic evaluation of dairy cattle using single-step test-day SNP-BLUP model

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Abstract

In many countries, single-step genomic models are replacing conventional pedigree-based models for routine valuation. Those models use all available information on the animals' phenotype, genotype, and pedigree. Pedigree data still has a huge impact on estimated genomic breeding values (GEBV), and it is also important to consider information about the structure of the pedigree. The foremost aspect of pedigree editing is dealing with missing parents' information. The choice of method of handling missing parents can affect the prediction of breeding values. This work investigates three scenarios of pedigree data: 1) Pedigree_real (**P_Real**) – pedigree from the routine evaluation, 2) Pedigree_2010 (**P_2010**) – at least 20 and 10 percent of dams and sires born before 2019 were set randomly to missing, respectively, 3) Pedigree_4020 (**P_4020**) – at least 40 and 20 percent of dams and sires born before 2019 were set randomly to missing, respectively. Moreover, for those pedigrees, three approaches to defining missing parents were used: 1) Raw pedigree (**RP**) – missing parents IDs set to missing, 2) Genetic groups (**GG**) – missing parents replaced by unrelated **GG**, which are defined based on year of birth, sex, and country of origin, 3) Metafounders (**MF**) – missing parents replaced by **MF**, which correspond to genetic groups. Relationships within and between metafounders were estimated from genomic information of descendants. The genomic breeding values for fat yield were estimated using the single-step test-day SNP-BLUP model, implemented by the MiXBLUP software. Although GEBV prediction was similar across scenarios, expressing missing parents by **GG** or **MF** impacts the genetic trend, especially in situations of limited pedigree completeness. Removing parent information led to reduced precision results across the methods of handling missing parents, since **P_Real** scenario demonstrated highest accuracy results. Compared to **RP** and **GG**, **MF** scenarios resulted in higher genetic trends. Insufficient pedigree completeness, especially among ungenotyped individuals, leads to an overestimation of the genetic trend. Completeness of pedigree information and a large number of genotyped individuals improve the reliability of evaluations. Modeling missing sires with **MF**s is less effective than assuming unrelated **GG**s if pedigree information is very incomplete. Therefore, the best method to model missing parents depends on completeness of pedigree.

Key words: single-step models, genetic groups, metafounders, validation

Introduction

The single-step model becomes the standard procedure of most national routine evaluations of dairy cattle (Legarra et al., 2014, Mäntysaari et al. 2017). The single-step model combines all available information, i.e., phenotype, genotype, and pedigree. Invariably, one of the main components in routine genomic evaluation of dairy cattle is the structure of the pedigree (Bradford et al., 2019). To reduce the bias due to missing information in the pedigree, genetic groups are used to associate individuals with missing parents with different categories (Westell et al., 1988, Legarra et al., 2007). An alternative to genetic groups to deal with missing information in the pedigree are the so-called metafounders (Legarra et al., 2015).

In this study, we focused on a single-step random regression SNP-BLUP test-day model for fat yield in the Polish Holstein population. The primary objective of this study is to evaluate various methods for handling missing parents and different levels of incompleteness in the pedigree data based on validation results, average GEBV trends, and GEBV comparisons.

Materials and Methods

This study is based on Polish national evaluation data for fat yield from April 2024 (Table 1). Two phenotype files were analysed: full data set – 63,615,019, and truncated data set – 58,446,695 test-day records. A truncated data set was created by removing the records for the youngest individuals, i.e., the last 4 years from the phenotype file. Genotypes that include 48,118 single-nucleotide polymorphisms (SNPs), were available for 113,019 cows and 68,972 bulls, that is 181,991 animals. The pedigree was extracted up to the third generation from animals with phenotypes and genotypes, including 4,712,143 animals (4,569,044 cows and 143,099 bulls).

Table 1: Number of test-day records, genotypes, and animals in the analysed data sets for fat yield.

Data	Sex	Number of animals	Number of records
Phenotype	Cows	3,707,727	63,615,019 Full data set 58,446,695 Truncated data set
Genotype	Cows	113,019	181,991
	Bulls	68,972	
Pedigree	Cows	4,569,044	4,712,143
	Bulls	143,099	

To deal with missing parents we used three approaches: 1) **RP** – raw pedigree with missing parents IDs set to missing; 2) **GG** – genetic groups with missing parents replaced by unrelated genetic groups, which are defined based on year of birth, country of origin and sex; 3) **MF** – metafounders with missing parents replaced by metafounders, which can be considered as genetic groups with relationships estimated from genomic information of descendants. Based on pedigree from routine evaluation, the three approaches of different pedigree completeness was used: 1) **P_Real** – pedigree from routine evaluation, with ~ 5.6% of missing sires and ~ 15.3% of missing dams; 2) **P_2010** – minimum 20% of dams and 10% of sires born before 2019 was set to missing based on **P_Real**; 3) P_4020 – minimum of 40% of dams and 20% of sires born before 2019 was set to missing based on **P_Real**. Only the parents' IDs were removed, as the manipulation involved animals born before 2019; therefore, the pedigree of the youngest validation animals remains the same across scenarios.

For animals with missing parents in the pedigree, the genetic groups were implemented based on country of origin, year of birth, and sex. Individuals born before 1961 were removed from the pedigree data. Over 70% of individuals included in the pedigree had both parents. Each genetic group contained a minimum of 20 animals. Group "-31" (Polish males born between 2010-2019) had the largest number of missing sires (1,002,069), whereas group "-32" (Polish females born between 2010-2019) had the most missing dams (174,954).The following single-step random regression test-day SNP-BLUP model (Liu et al., 2004; Liu et al., 2014) was applied:

$$y=Xh+Wf+Vp+Vu+e,$$

where $\boldsymbol{y}$ is a vector of cows' test day records for fat yield from the first three lactations, $\boldsymbol{h}$ is a vector of fixed effects of herd-test-day-parity-milking frequency, $\boldsymbol{f}$ is a vector of fixed lactation curve coefficients which was modelled by the Wilmink function (Liu et al., 2004), $\boldsymbol{p}$ is a vector of permanent environmental effects expressed as random regression coefficient coefficients of the Legendre polynomials, $\boldsymbol{u}$ is a random additive genetic effects also described by the random regression coefficients of the Legendre polynomials.

The GEBVtest method was used for validation (Mäntysaari et al., 2010). The full and truncated data sets have been prepared for validation. The full data set contains all phenotypic data, while the truncated data set includes all phenotypic data except for the last 4 years of data. Validation cows were defined as cows whose records were removed for a truncated data set; however, validation bulls were defined as sires born between 2017 and 2019, and having more than 20 validation daughters. The test was implemented separately for validation cows and bulls, used the linear regression:

$$\mathbf{GEBVf} = b_0 + b_1\mathbf{GEBVp} + \mathbf{e},$$

where **GEBVf** represents the vector of GEBVs predicted based on the full data set, while **GEBVp** represents GEBVs predicted based on the truncated data set, b_0 represents the intercept, which indicates a systematic bias in the model's prediction, and b_1 represents the regression slope, the dispersion of prediction compared to actual results. The R^2 coefficient is one of the results of linear regression and serves a measure of prediction accuracy, it indicates the percentage of variance in the **GEBVp** explained by **GEBVf**.

Validation results were computed for the first three lactations, and the total genomically enhanced breeding value (GEBV) defines as:

$$GEBVt=0.5GEBV_1+0.3GEBV_2+ 0.2GEBV_3$$

where $GEBV_1$ is GEBV for the 1_{st} lactation, $GEBV_2$ is GEBV for the 2_{nd} lactation and $GEBV_3$ is GEBV for the 3_{rd} lactation.

Single-step genomic evaluations were conducted using MiXBLUP 3.0 (Vandenplas et al., 2022)

Results & Discussion

Validation results are reported for 482,810 validation cows and 562 validation bulls. Figures 1-3 show validation results for all scenarios divided by sex, method, and genotyping status. Figure 1 shows the b_0 of the dam and sire. We observed similar results for all scenarios; the values are close to 0, which is expected. Figure 2 shows the b_1 value, which is similar for every scenario for validation cows, with b_1 in the range of 0.96 (**P_Real MF** ungenotyped) to 1.1 **(P_2010 MF** genotyped). However, for validation bulls, all results are similar, except for ungenotyped validation bulls in the scenarios **P_4020** and **P_2010** for **MF**. For these latter categories, we observed an overestimation of b_1 at 1.27 (**P_2010**) and 1.33 **(P_4020**). This may be due to a lack of pedigree connection for ungenotyped bulls, due to a higher percentage of incomplete pedigrees. Figure 3 shows the R^2, ranging from 0.66 to 0.90 for every scenario. Lower values were observed for ungenotyped validation cows; however, for genotyped validation cows, R^2 is more stable and similar across scenarios. For ungenotyped validation bulls, we observed a trend where R^2 increased from **RP** through **GG** and **MF**. However, for genotyped validation bulls, the R^2 value is similar for **P_Real**. In contrast, for MF, the R^2 values for **P_4020** and **P_2010** are lower than in other scenarios involving missing parents.

Figure 4 compares full and truncated data sets for validation bulls divided by scenarios and genotyping status. In each case, the points cluster together to form an extended cloud centered on the diagonal; however, as parental information is gradually eliminated, the cloud dispersion becomes wider, especially for ungenotyped individuals. The effect is slight under **P_Real**, becomes evident in **P_2010**, and reaches its peak in **P_4020**, when ungenotyped validation bulls from the **RP**, **GG**, and **MF** deviate the most from the diagonal. All of these patterns show that genomic information protects the accuracy of prediction when the incompleteness of pedigree is high: prediction for genotyped validation bulls remains strong even when up to 40% of dams and 20% of sires are set to unknown, whereas missing parental information links weaken the stability of GEBV for ungenotyped validation bulls.

Figure 5 shows the average GEBV trend for all scenarios divided by sex. Since 2000, the mean GEBV has increased gradually; however, after 2010, when genotyping became widely used in Poland, the increase became more pronounced. Compared to cows, bulls exhibit a steeper trajectory, indicating that the sire pathway is under more selection pressure. Both sexes show the same scenario ranking, with **MF** producing the highest averages, followed by **GG** and **RP**. However, as pedigree completeness declines, the gap between scenarios widens, underscoring the fact that the way missing parents are handled can significantly skew the perception of genetic progress. It is crucial to handle incomplete pedigrees robustly to prevent overestimating or underestimating the selection response.

Conclusions

The results demonstrate that the method used to close pedigree gaps can significantly affect the predictions of GEBV. Regardless of the pedigree scenario used, the real pedigree yielded the most reliable validation results. However, for individuals without genotypes, scenarios with increased pedigree incompleteness introduced observable over-dispersion; this effect was more pronounced for sires than for dams and was most noticeable in the **MF** group.

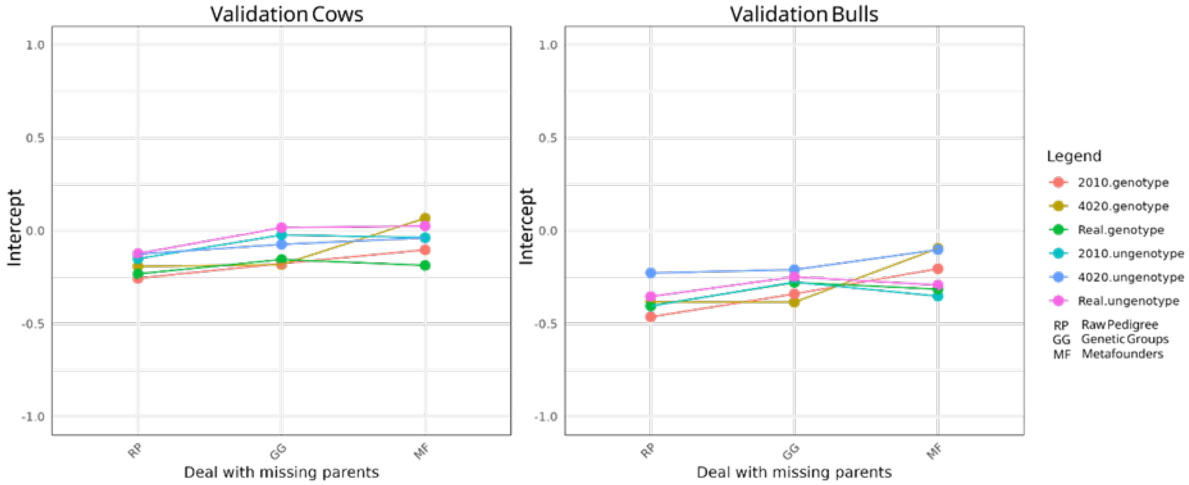

Figure 1. Intercept (b_0) for validation individuals divided by sex and method.

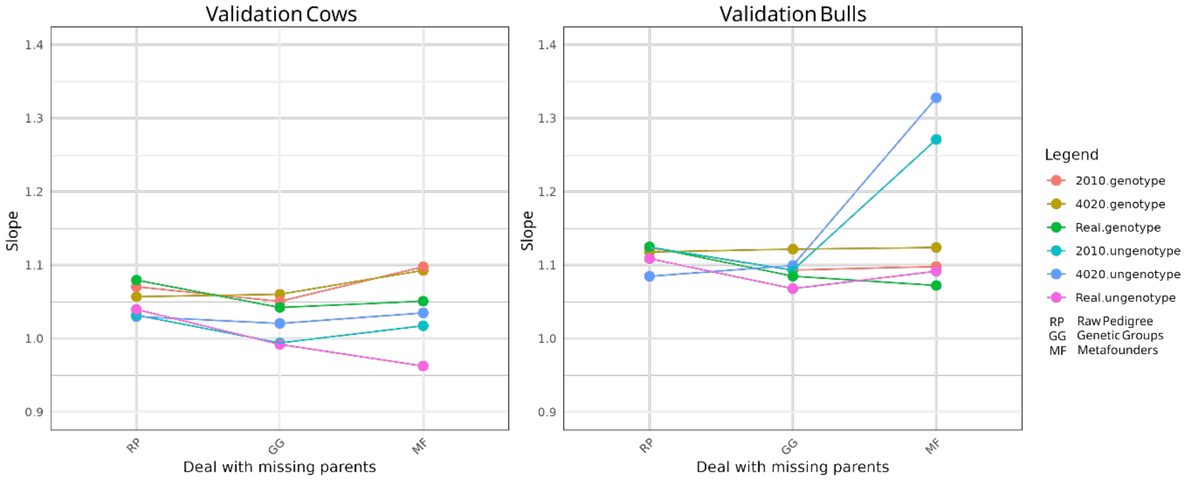

Figure 2. Slope (b_1) for validation individuals divided by sex and method.

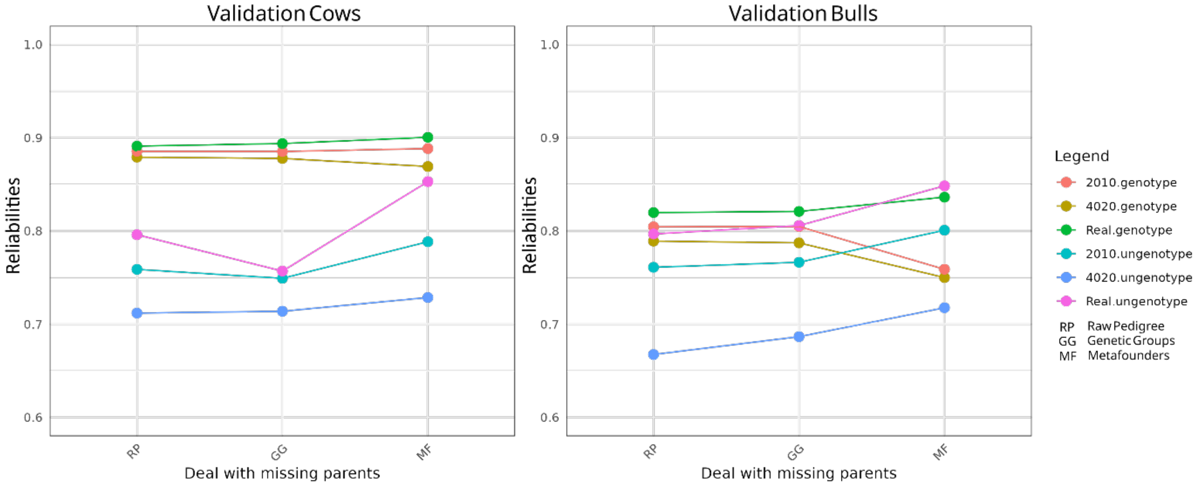

Figure 3. Reliabilities (R^2) for validation individuals divided by sex and method.

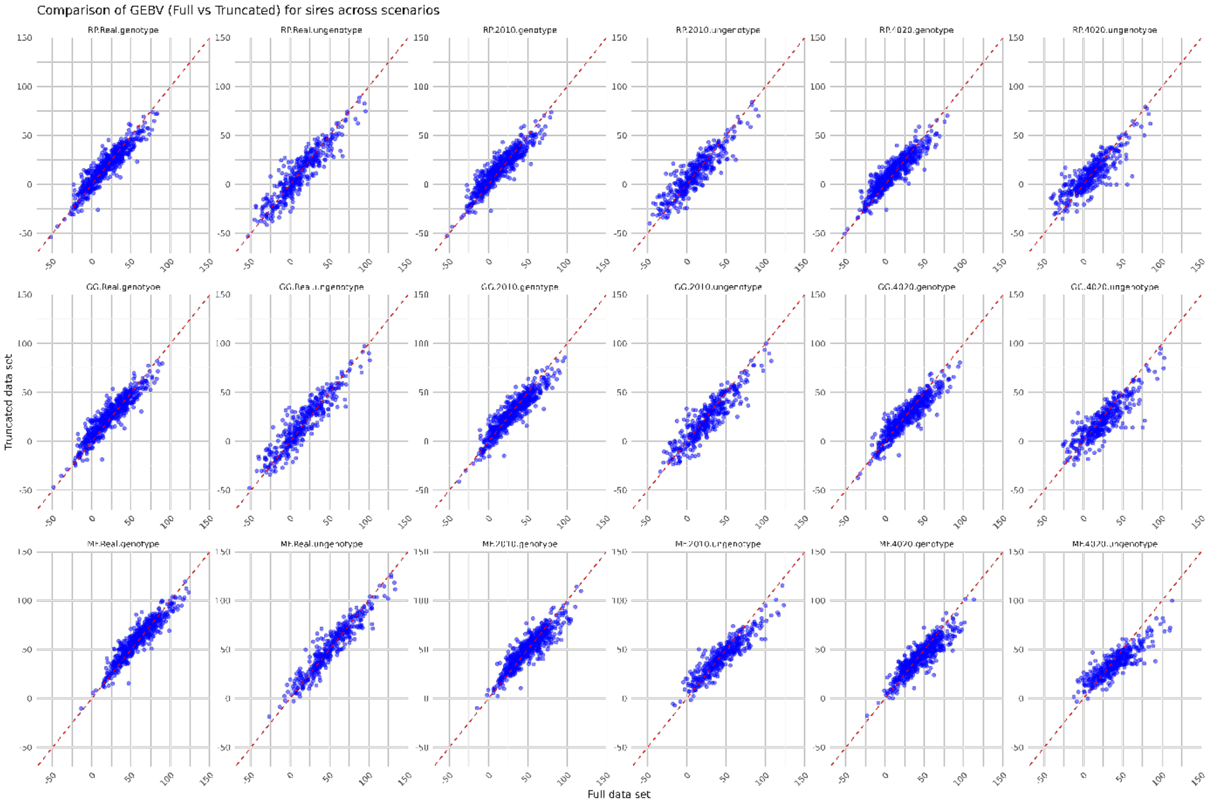

Figure 4. Comparison of GEBV for validation bulls across scenarios, divided by genotyped and ungenotyped individuals.

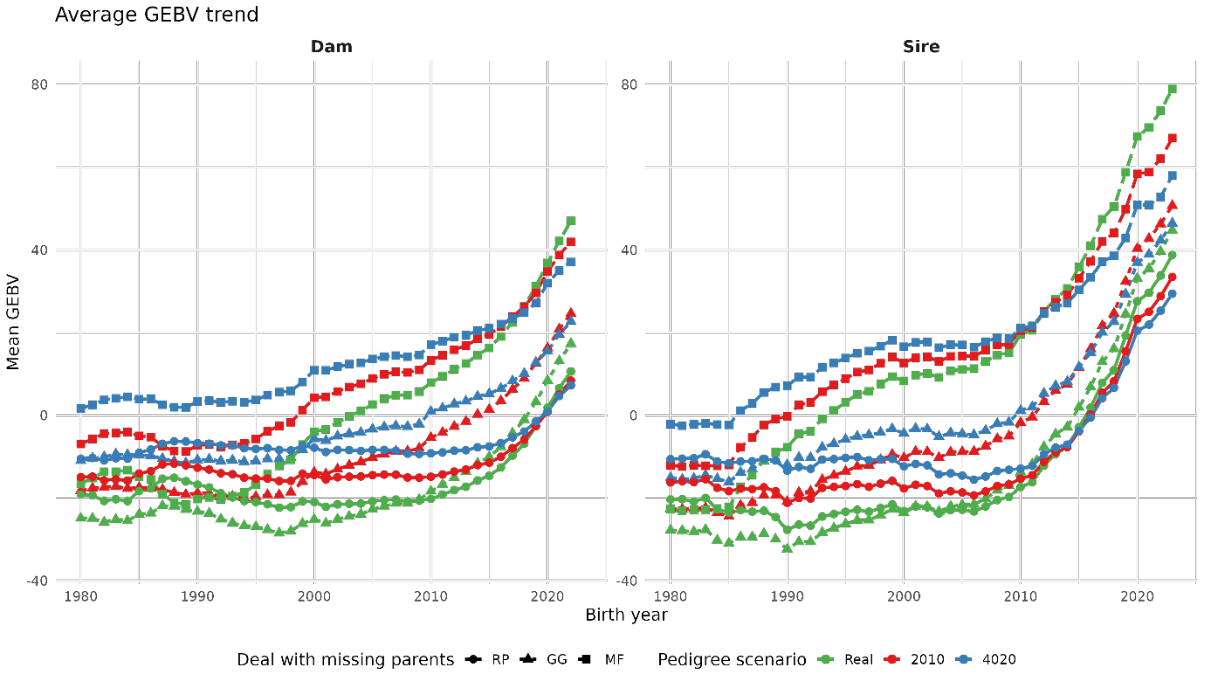

Figure 5. Average GEBV trend divided by sex and method.

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Integration of single step DGV in conventional genetic evaluations using DGV-PBLUP

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Abstract

Routine single-step genomic evaluations can be costly in time and computer resources. Hence, newly genotyped animals initially receive a genomic prediction of their direct genomic values (DGV). If genomic predictions of DGVs of animals become available, it may be convenient to estimate GEBV of such animals using some form of integration into conventional pedigree BLUP evaluations. DGV-PBLUP is a novel method of integration of DGV from genomic predictions, into a conventional pedigree BLUP (PBLUP) evaluation. This is done by setting the prior mean of the animal genetic effect (which usually is zero in linear mixed models) to the DGV to be incorporated. In this paper we report on the application of this methodology to the Dutch-Flemish genetic evaluation. Results showed a high correlation (0.99 or higher) between GEBV of animals associated with a genotype in single step SNP-BLUP and a GEBV in DGV-PBLUP. Run time of DGV-PBLUP evaluations were comparable to conventional pedigree evaluations and much shorter than routine single-step SNP-BLUP evaluations. DGV-PBLUP promises to be a convenient method of integration of genomic information into pedigree BLUP evaluations, without the need for sharing or accessing SNP genotypes.

Key words: Single-step, pedigree BLUP, integration, genomic evaluations

Introduction

Routine single-step evaluations can be costly in time and computer resources. Hence, newly genotyped animals initially receive a genomic prediction of their direct genomic values (DGV). If for some animals genomic predictions of DGV become available it still may be convenient to compute their GEBV using some form of integration into conventional pedigree BLUP evaluations.

There may also be cases where only DGV of animals are available for evaluation, without genotype data, due to legal or legislative considerations. This is the case at CRV, which consists of a commercial half, the corporation, and a cooperative half, with dairy farmer membership. The cooperative publishes national genetic evaluations. However, the single step evaluation is corporately owned. For reasons of IP protection, the corporation cannot share genotypes or allele substitution effects. The cooperative and the corporation have entered in an agreement, where the corporation supplies the cooperative with DGV for inclusion in the national evaluation. If such DGV are to be used in national genetic evaluations, integration is still required.

Integration of genomic data into genetic evaluations has been a long standing subject in the field of animals quantitative genetics and breeding. Methods of integration saw an evolution from linear post-processing after evaluation, via methods using pseudo-records during evaluation, where DGV are fitted as observations on a pseudo-trait added to the evaluation and correlated to the target trait (Stoop *et al.*; 2014) to single-step models, where genotypes are fitted in the statistical model of evaluations. Integration methods of genomic information were successful in achieving their stated goals, but true equivalency between such methods and single-step evaluations were not achieved.

In this paper we present a model of integration that is mathematically equivalent to single-step SNP-BLUP (ssSNPBLUP) models, but only requires DGV of genotyped animals, in addition to conventional phenotypic and pedigree data.

Materials and Methods

Model

The equations of the model were derived from the ssSNPBLUP linear equations proposed by Liu et al. (2014). If we assume that estimates of SNP effects $\hat{\mathbf{g}}$ are known before performing a single-step genomic prediction, then the vector **d** with predicted DGV of genotyped animals can be computed as **d** = **Zg**, where **Z** is the genotyped matrix centered with observed allele frequencies, and we can assume the following prior multivariate normal (MVN) distribution for the genetic additive effects **u**:

$$[\mathbf{u}|\hat{\boldsymbol{\mu}}, \mathbf{A}^*] \sim MVN(\hat{\boldsymbol{\mu}}, \mathbf{A}^*\sigma_u^2)$$

with

$$\hat{\boldsymbol{\mu}} = \begin{bmatrix} \mathbf{A}_{ng}\mathbf{A}_{gg}^{-1} \\ \mathbf{I} \end{bmatrix} \mathbf{d}$$

and

$$\mathbf{A}^{*-1} = \begin{bmatrix} \mathbf{A}^{nn} & \mathbf{A}^{ng} \\ \mathbf{A}^{gn} & \mathbf{A}^{gg} + \left(\frac{1}{w} - 1\right)\mathbf{A}_{gg}^{-1} \end{bmatrix},$$

where the subscripts n and g refer to ungenotyped and genotyped animals, respectively,

$$\mathbf{A}^{-1} = \begin{bmatrix} \mathbf{A}_{nn} & \mathbf{A}_{ng} \\ \mathbf{A}_{gn} & \mathbf{A}_{gg} \end{bmatrix}^{-1} = \begin{bmatrix} \mathbf{A}^{nn} & \mathbf{A}^{ng} \\ \mathbf{A}^{gn} & \mathbf{A}^{gg} \end{bmatrix}$$

is the inverse of the pedigree relationship matrix partitioned between genotyped and ungenotyped animals, w is the proportion of additive genetic variance explained by the residual polygenic effects, σ_u^2 is the genetic variance, **d** is the vector with DGV of genotyped animals, and **I** is an identity matrix.

The system of equations associated with these assumptions, hereafter called DGV-PBLUP, is written as follows:

$$(1) \begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{A}^{*-1}\sigma_u^{-2} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{\beta}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} + \mathbf{A}^{*-1}\sigma_u^{-2}\hat{\boldsymbol{\mu}} \end{bmatrix}$$

where $\hat{\boldsymbol{\beta}}$ is the vector of estimated fixed effects, $\mathbf{y}$ is the vector of records, $\mathbf{R}^{-1}$ is the inverse of the residual variance structure matrix, and **X** and **Z** are incidence matrices relating records to the fixed and additive genetic effects, respectively.

The system of equations of DGV-PBLUP is equivalent to a single-step genomic evaluation, provided that the SNP effects $\hat{\mathbf{g}}$ were estimated using the same phenotypic, genomic and pedigree information (Vandenplas et al, 2021). The system of equations of DGV-PBLUP can also be considered as an application of the Bayesian procedure to integrate external information into genetic evaluations (Legarra et al., 2007; Vandenplas and Gengler, 2012), where, in essence a prior mean is fitted for all animals, based on the (imputed) DGV of (un)genotyped animals.

Data

The DGV-PBLUP method was tested on a dataset and associated variance components of the milk production test day model (TDM), which is a 5 lactation, 4th order random regression with Legendre polynomials (5 regressions per lactation), analyzing milk, fat, protein and lactose yield, as well as somatic cell score and urea content of milk.

Phenotypic data were taken from the April 2025 evaluation of CRV. DGV for each of the 25 regressions were taken from a genomic prediction based on SNP effect estimates from a single-step SNPBLUP evaluation on the same phenotypic data (April '25). The latter included 828,590 genotyped animals. The data in the DGV-PBLUP evaluation consisted of 16,382,568 pedigreed animals, 13,662,463 of which had phenotypic data. Also included were DGV of 851,704 animals.

GEBV from DGV-PBLUP were compared to results from the corresponding single-step SNPBLUP run. GEBV from the current pseudo-record evaluation (Stoop et al., 2014) were also contrasted to these. GEBV were produced for the following traits or trait groups:

1) Milk production (lac. 1-5 and overall)
2) Fat production (lac. 1-5 and overall)
3) Protein production (lac. 1-5 and overall)
4) Lactose production (lac. 1-5 and overall)
5) Somatic cell score (lac. 1-5 and overall)
6) Urea content (lac. 1-5 and overall)

Presented in this paper are the comparison of GEBV from ssSNPBLUP and DGV-PBLUP for young bulls without progeny, born after 2020, since this group of animals is the most sensitive to changes in genomic information in an evaluation. For the overall traits the Pearson correlation were calculated, as well as the fraction of animals whose GEBV differed less than a quarter genetic standard deviation, as an indication of GEBV stability. For reference the same statistics were produced from the current pseudo-record (PSR) method of integrating genomic information into the national evaluation.

Results & Discussion

Breeding values

A comparison of the GEBVs from DGV-PBLUP and the current PSR system for overall GEBV of traits in the milk production test-day random regression model are presented in Table 1. Correlations with ssSNPBLUP GEBV were clearly improved with DGV-PBLUP, with all correlations > 0.99. Changes in GEBV from ssSNPBLUP to integrated GEBV were also considerably smaller for DGV-PBLUP, with virtually all GEBV with ¼ genetic standard deviation. This also indicates a considerable improvement in GEBV stability compared to the PSR system, where the fraction of animals changing more than ¼ s.d. was considerably larger.

An attractive feature of the DGV-PBLUP method is that no extra correlated traits have to be fitted to incorporate DGV information in a pedigree BLUP evaluation. Neither does it require a post-processing step to integrate DGV.

Table 1. Number of selected bulls, correlations with ssSNPBLUP GEBV and fraction of animals whose GEBV changed less than ¼ genetic standard deviation for the DGV-PBLUP method (*dgv*) and the current pseudorecord method of integration (*psr*).

Trait		*Correlation*		*< 1/4 s.d.*	
	N	*dgv*	*psr*	*dgv*	*psr*
Milk	5,629	0.999	0.928	100.0%	72.2%
Fat	5,629	0.999	0.964	100.0%	80.1%
Protein	5,629	0.998	0.934	100.0%	74.8%
Lactose	5,629	0.995	0.972	99.8%	85.3%
SCS	5,629	0.999	0.994	100.0%	98.3%
Urea	5,629	0.999	0.918	100.0%	70.5%

Selected were young bulls without progeny born after 2020.

Table 2. Run times of genetic evaluations of the milk production test day model. Run times are given in hours:minutes for routine ssSNPBLUP evaluations, DGV-PBLUP and conventional pedigree BLUP evaluations.

Trait	*ssSNPBLUP*	*DGV-PBLUP*	*PBLUP*
Milk	51:28	18:17	17:53
Fat	47:26	18:39	17:37
Protein	49:05	19:20	18:08
Lactose	48:47	19:02	19:30
SCS	56:02	18:22	18:31
Urea	63:00	18:49	19:45

All evaluations were run using 5 threads for parallel computing on a server with Intel(R) Xeon(R) Gold 6448H 64bit chips at 4000MHz.

Run time

The wall clock times of all evaluations are presented in Table 2. All evaluations were run without starting values. The run times of DGV-PBLUP were comparable to the run times of conventional pedigree BLUP evaluations, as expected. The run times of routine single-step SNPBLUP evaluations on average were 2.4 times longer than either conventional or DGV-PBLUP evaluations.

Conclusions

DGV-PBLUP presents itself as a superior method of integrating genomic data into conventional pedigree BLUP evaluations, in the sense that it replicates more closely the results of a routine single-step SNPBLUP run than the PSR method of integration currently implemented at CRV. DGV-PBLUP promises to be a convenient method of integrating genomic information into pedigree BLUP evaluations, without the need for sharing SNP genotypes.

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Lazy computation of Residual Polygenic effects

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Abstract

Single step methods use a blended relationship matrix that contains a fraction (typically 5%-20%) of pedigree-based relationships, called Residual Polygenic Effect (RPG). Indirect predictions of animals not included in the Mixed Model Equations (MME) are composed by a Direct Estimated Genomic Value (DGV), a sum of SNP readings times their solutions, and an RPG. Computation of RPG is not straightforward, and involves some complicated algebra and software, including separate relationships for genotyped and non-genotyped animals. We propose an alternative, equivalent computation that infers RPG for genotyped animals in the MME as RPG=GEBV-DGV, and then solves BLUP equations with RPG as "record" with heritability close to 1. The solution is the RPG for all animals in the MME, from which Parent Average can be used for Indirect Predictions. We show feasibility in practice with a US data set with millions of animals genotyped and in pedigree.

Key words: single step, residual polygenic effects

Introduction

Genomic evaluations typically include a fraction of pedigree-based relationships usually called Residual Polygenic effect (RPG). This fraction considers the relationships not well covered by markers, regress the genomic relationships towards pedigree ones, and generally prevents the evaluations from overdispersion (VanRaden, 2008; Liu et al., 2011). The resulting final GEBV $\hat{\mathbf{u}} = \hat{\mathbf{u}}^*$ can be separated into genomic-based and pedigree-based parts that we will call DGV (Direct Genomic Value). For genotyped animals g this is equal to the sum of SNP solutions $\hat{\mathbf{u}}_g^* = \mathbf{Z}\hat{\mathbf{a}}$, and the RPG part that is $\hat{\mathbf{d}}_g = \mathbf{A}_{g,g}\mathbf{G}^{-1}\hat{\mathbf{u}}_g$ (Legarra and Ducrocq, 2012, eq [8]).

RPGs are conceptually transmitted as an infinitesimal trait and therefore their covariances are modelled using pedigree-based relationships $\mathbf{A}$. The RPG of non-genotyped animals n as a function of genotyped ones can be obtained using some equivalent expressions (Vandenplas et al. 2023) which involve dedicated programming, among them $\hat{\mathbf{d}}_n = \mathbf{A}_{ng}\mathbf{A}_{gg}^{-1}\hat{\mathbf{d}}_g$ which solves RPG for non-genotyped animals.

New animals with genotypes (selection candidates) are typically evaluated, at least at first, based on solutions from the previous Single-step run. The DGV part is easily computed from the newly read genotype $\boldsymbol{z}$ as $\hat{u}^* = \boldsymbol{z}\hat{\boldsymbol{a}}$. The RPG part can be obtained as Parent Average of RPG from the ancestors, proceeding in pedigree order if needed from animals in the Single Step equations. This needs all solutions of RPG for non-genotyped animals, e.g. from $\hat{\mathbf{d}}_n = \mathbf{A}_{n,g}\mathbf{A}_{g,g}^{-1}\hat{\mathbf{d}}_g$.

The last equation can be a bit cumbersome to obtain, and Vandenplas et al. (2023) propose a few equivalent expressions, which need to be programmed. Here we propose an alternative shortcut that uses BLUP to obtain $\hat{\mathbf{d}}_n$ and therefore "regular" BLUP solvers can be used.

Materials and Methods

We arrange all animals (and Unknown Parent Groups or Metafounders) in the single step evaluation into non-genotyped and genotyped animals. Consider the following MME:

$$\begin{pmatrix} \mathbf{A}^{n,n}\lambda & \mathbf{A}^{n,g}\lambda \\ \mathbf{A}^{g,n}\lambda & \mathbf{A}^{g,g}\lambda + \mathbf{I} \end{pmatrix} \begin{pmatrix} \hat{\mathbf{d}}_n \\ \hat{\mathbf{d}}_g^* \end{pmatrix} = \begin{pmatrix} 0 \\ \hat{\mathbf{d}}_g \end{pmatrix} \quad [1]$$

For $\lambda = \frac{1-h^2}{h^2}$. When $h^2 \to 1$, $\lambda \to 0$ and $\hat{\mathbf{d}}_g^* \approx \hat{\mathbf{d}}_g$. From the top equation we obtain $\hat{\mathbf{d}}_g = -(\mathbf{A}^{n,n})^{-1}\mathbf{A}^{n,g}\hat{\mathbf{d}}_g = \mathbf{A}_{n,g}\mathbf{A}_{g,g}^{-1}\hat{\mathbf{d}}_g$. In other words, we obtain RPG solving BLUP equations.

Convergence of this iterative system is slow, as it will be shown later, because there are many more non-genotyped than genotyped animals. Thus, a second, approximated model and associated MME are:

$$\begin{pmatrix} \mathbf{1}'\mathbf{1} & \mathbf{0} & \mathbf{1}' \\ \mathbf{0} & \mathbf{A}^{n,n}\lambda & \mathbf{A}^{n,g}\lambda \\ \mathbf{1} & \mathbf{A}^{g,n}\lambda & \mathbf{A}^{g,g}\lambda + I \end{pmatrix} \begin{pmatrix} \hat{\mu} \\ \hat{\mathbf{d}}_n \\ \hat{\mathbf{d}}_g^* \end{pmatrix} = \begin{pmatrix} \mathbf{1}'\hat{\mathbf{d}}_g \\ \mathbf{0} \\ \hat{\mathbf{d}}_g \end{pmatrix} \quad [2]$$

upon solution of this system, we should add back $\hat{\mu}$ to $\hat{d}_g^*$ and $\hat{d}_n$.

After running a single step evaluation with US data, at CDCB we tested both [1] and [2] with the “yield” group of traits (milk, fat and protein yields) with ~50M animals in pedigree, ~2.5M animals genotyped. Therefore there are 2.5M RPG “records” (obtained as $\hat{\mathbf{d}}_g = \hat{\mathbf{u}}_g - \mathbf{Z}\hat{\mathbf{a}}$) and 48M animals with “no records”, for a total of 150M equations. We used PCG iteration in blup90iod3 from the BLUPF90 suite (Lourenco et al., 2022), with 8 threads. We tried several convergence criteria from 10^{-6} to 10^{-14}, and we considered 10^{-14} as pseudo-true. Then we computed the correlation with the pseudo-true solutions.

In addition, we verified number of rounds needed to achieve PCG convergence of 10^{-12} for all traits evaluated by CDCB except calving ease and stillbirth.

Results & Discussion

Results for yield traits are presented in Tables 1 and 2. Time per round is 20 seconds so 1000 iterations take ~5h, and this is a post-processing step after the single step run that need not be run again. For model [1], the convergence with the pseudo-true solution is quite fast, with a correlation of almost 1 at a PCG convergence of 10^{-10}.

On the other hand, model [2] is of faster apparent convergence but of actual slower one – good correlations with the pseudo-true solution appear at PCG convergence levels of 10^{-14} and smaller. So the total computing time is about the same, and the convergence is more misleading. Model [2] is not recommended.

Table 3 shows number of rounds to reach PCG convergence of 10^{-12}. Trait groups that take longest time (over 1,000 iterations) are those with large number of animals and lowest proportion of animals genotyped. For instance, “Fertility” has 50M animals in pedigree, 2.2M of them genotyped; “Health” has ~10M animals in pedigree, 1.2M of them genotyped; and “Residual Feed Intake” has 60K animals in pedigree, 9K of them genotyped.

Table 1: correlation of the solution for RPGs using [1] with the pseudo-true solution, yield traits.

PCG convergence	correlation with pseudo-true	iteration
10E-06	0.916	113
10E-07	0.958	244
10E-08	0.978	381
10E-09	0.995	655
10E-10	0.9995	898
10E-11	0.999988	1177
10E-12	0.999999	1385
10E-13	1	1549
10E-14	1	1808

Table 2: correlation of the solution for RPGs using [2] with the pseudo-true solution, yield traits

PCG convergence	correlation with pseudo-true	iteration
10E-10	0.872	31
10E-11	0.895	46
10E-12	0.914	79
10E-13	0.950	213
10E-14	0.995	568
10E-15	0.9992	712
10E-16	0.9996	804

Table 3: rounds to reach PCG convergence of 10E-12, all trait groups

Trait group	iteration
Fertility	1476
Gestation length	384
Heifer livability	460
Health	397
Livability	1751
Productive life	1537
Residual feed intake	104
Somatic cell score	1040
Yield	1385

Conclusions

In absence of dedicated software, the RPG solutions for animals in the single step equations can be computed using BLUP with $h^2 \approx 1$. Computing time is a few hours and it depends on the ratio genotyped animals/non-genotyped animals. This BLUP gives a simple and competitive solution to backsolve RPGs for all animals considered in the evaluation. Those RPGs can be used later for candidates to selection through “indirect predictions”.

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Comparing methods for approximating reliabilities in large-scale single-step genomic evaluations

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Abstract

Accurate approximation of genomic estimated breeding value (GEBV) reliabilities is vital in single-step genomic prediction as reliable predictions of GEBV facilitate effective selection decisions. However, calculating exact reliabilities by inverting the left-hand side matrix of the mixed model equations is computationally infeasible for large datasets. In this study, we compared two approaches from Luke and Interbull for approximating genomic reliabilities for both genotyped and non-genotyped animals. The Luke approach uses effective record contributions (ERC) derived from the conventional EBV reliabilities as weights to approximate GEBV reliabilities for genotyped animals. A blended approach is used to implicitly account for residual polygenic (RPG) effects. Subsequently, genomic information is propagated to non-genotyped animals using ERC weights derived from the reliabilities of the genotyped animals. In contrast, the Interbull approach requires the derivation of a constant parameter, denoted φ_c, which is the genomic effective daughter contribution (EDC) gain via the Interbull GEBV test. This parameter is used to propagate genomic information to non-genotyped relatives through the pedigree. The final genomic reliabilities are obtained by combining conventional reliabilities with the genomic reliability gain. Notably, accuracy of reliabilities by this method highly depends on the precise estimation and regular updating of φ_c. In addition, this approach requires validation-based adjustments to correct inflated theoretical reliabilities observed in extremely large reference populations. In this study, both approaches were assessed and compared against exact reliabilities using a real dataset from the Finnish Red dairy population under a single trait model. The results demonstrated that the approximated reliabilities from both approaches were in close agreement with the exact reliabilities. Thus, both approaches can offer effective strategies for obtaining the reliabilities of GEBV in practical large-scale single-step evaluations.

Key words: single-step model, GEBV reliability, SNPBLUP, EDC, ERC

Introduction

Single-step methods (Legarra et al., 2009, Christensen and Lund, 2010) allow computing genomic estimated breeding values (GEBV) for both the genotyped and non-genotyped individuals simultaneously. Their adoption in routine genetic evaluations has become increasingly widespread in dairy cattle breeding. Consequently, the accurate computation of GEBV reliabilities has gained importance for supporting effective selection decisions. However, computing exact reliabilities by inverting the left-hand side of the mixed model equations (MME) becomes computationally infeasible for large-scale datasets. Thus, efficient approximation methods are needed.

Several methods for approximating the reliabilities of GEBV have been proposed and implemented (Misztal et al., 2013, Edel et al., 2019, Ben Zaabza et al., 2022, Bermann et al., 2022, Gao et al., 2023). In particular, to ensure the international comparability of national genomic reliabilities, an Interbull working group was established in 2016 to develop a standardized procedure for estimating GEBV reliabilities in dairy cattle genetic evaluations (Liu et al., 2017). A corresponding guideline targeting large-scale genotyped populations has recently been released (Liu et al., 2024).

In this study, we compared two approaches for approximating genomic reliabilities for both genotyped and non-genotyped animals. The first approach, hereafter referred to as the Luke approach, uses effective record contributions (ERC) as weights within simplified SNPBLUP and PBLUP models to approximate GEBV reliabilities (Gao et al., 2023). The second approach, hereafter referred to as the Interbull approach, combines the genomic reliability gain with the conventional EBV reliability to obtain the final GEBV reliability for all animals (Liu et al., 2024).

Materials and Methods

The Luke approach

This is a three-step approach to approximate GEBV reliabilities in a single-step model that includes a residual polygenic (RPG) effects (Gao et al., 2023).

Step 1: Compute reliabilities of direct genomic values (DGV) for the genotyped animals

A simplified single-trait weighted SNPBLUP without RPG effects was used:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Z}\mathbf{g} + \mathbf{e} \qquad (1)$$

where $\mathbf{y}$ is an $n \times 1$ vector of (pseudo) phenotypes; μ is the general mean; $\mathbf{1}$ is an $n \times 1$ vector of ones; $\mathbf{Z}$ is an $n \times m$ matrix of SNP marker covariates centered and scaled using VanRaden method 1 (VanRaden, 2008), $\mathbf{g}$ is an $m \times 1$ vector of the SNP marker effects; $\mathbf{e}$ is a vector of residuals. It is assumed that $\mathbf{g} \sim N(\mathbf{0}, \mathbf{I}_m\sigma_u^2)$, and $\mathbf{e} \sim N(\mathbf{0}, \mathbf{D}_n^{-1}\sigma_e^2)$, where $\mathbf{D}_n$ is a diagonal matrix with elements d_{ii} equal to the ERC_i value for genotyped animal i, computed by reversing the method of Tier and Meyer (2004) using the conventional EBV reliabilities for the genotyped animals, and σ_u^2 and σ_e^2 are the additive genetic and the residual variances, respectively. The MME for model (1) is:

$$\begin{bmatrix} \mathbf{1}'\mathbf{D}_n\mathbf{1} & \mathbf{1}'\mathbf{D}_n\mathbf{Z} \\ \mathbf{Z}'\mathbf{D}_n\mathbf{1} & \mathbf{Z}'\mathbf{D}_n\mathbf{Z} + \lambda\mathbf{I}_m \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{1}'\mathbf{D}_n\mathbf{y} \\ \mathbf{Z}'\mathbf{D}_n\mathbf{y} \end{bmatrix} \qquad (2)$$

with $\lambda = \frac{\sigma_e^2}{\sigma_u^2}$. We partitioned and denoted the inverse of the LHS matrix of the MME as $\begin{bmatrix} \mathbf{C}^{\mu\mu} & \mathbf{C}^{\mu\mathbf{g}} \\ \mathbf{C}^{\mathbf{g}\mu} & \mathbf{C}^{\mathbf{gg}} \end{bmatrix}$. The reliability of DGV for genotyped animal i is $r_{g,g,i}^{2*} = 1 - \lambda\frac{\mathbf{Z}_i\mathbf{C}^{\mathbf{gg}}\mathbf{Z}_i'}{\mathbf{G}_{ii}}$, where $\mathbf{Z}_i$ represents row i in $\mathbf{Z}$, and $\mathbf{G}_{ii}$ is the diagonal element i of the genomic relationship matrix $\mathbf{G} = \mathbf{Z}\mathbf{Z}'$.

Note that the RPG effects were not explicitly included in model (1) to preserve the dimensionality and computational advantages of SNPBLUP model, particularly in scenarios where the number of individuals (n) greatly exceeds the number of markers (m).

The RPG effects were accounted for by blending the above DGV reliabilities with the traditional EBV reliabilities:

$$r_{g,g,i}^2 = \frac{(1-\omega)\mathbf{G}_{ii}r_{DGV,i}^2 + \omega\mathbf{A}_{22\,ii}r_{EBV,i}^2}{(1-\omega)\mathbf{G}_{ii} + \omega\mathbf{A}_{22\,ii}} \qquad (3)$$

where $\mathbf{A}_{22}$ is the submatrix of $\mathbf{A}$ corresponding to the genotyped animals, $\mathbf{A}_{22\,ii}$ is the diagonal element i of the $\mathbf{A}_{22}$ matrix which is equal to $1+F_i$ with F_i equal to the pedigree-based inbreeding coefficient of animal i,; $r_{DGV,i}^2$ is the DGV reliability for animal i and $r_{EBV,i}^2$ is the EBV reliability for animal i. ω is the proportion of the RPG effects.

Step 2: Calculate the genomic ERC for the genotyped animals

The ERC accounting for the genomic information for all genotyped animals can be calculated as:

$$ERC_g = ERC_{conv} + \frac{1-h^2}{h^2}\left(\frac{r_{DGV}^2}{1-r_{DGV}^2} - \frac{r_{EBV}^2}{1-r_{EBV}^2}\right) \qquad (4)$$

where ERC_{conv} is the conventional ERC for the genotyped animals. Note that these genomic ERC values are included as weights for the genotyped animals when computing the GEBV reliabilities for non-genotyped animals in Step 3.

Step 3: Compute reliabilities of GEBV for the non-genotyped animals

A simplified single-trait weighted PBLUP model was used:

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{a} + \mathbf{e} \tag{5}$$

where **y** is a $p \times 1$ vector of pseudo phenotypes with p equal to the number animals in the pedigree; µ is the general mean; **1** is a $p \times 1$ vector of ones; **a** represents a $p \times 1$ vector of additive genetic effects; **e** is a vector of residuals. It is assumed that $\mathbf{a} \sim N(\mathbf{0}, \mathbf{A}\sigma_u^2)$ and $\mathbf{e} \sim N(\mathbf{0}, \mathbf{D}_p^{-1}\sigma_e^2)$, where **A** is the numerator relationship matrix and $\mathbf{D}_p$ is a diagonal matrix with elements of ERC from vector of $\begin{bmatrix}\mathbf{ERC}_{conv}\\ \mathbf{ERC}_g\end{bmatrix}$, and σ_u^2 and σ_e^2 are the additive genetic and residual variances, respectively.

The Interbull approach

This approach is a three-step approach which requires the Interbull GEBV test (Mäntysaari et al., 2010), thus it has been feasible for routine single-step genomic evaluation with millions of genotyped animals (Liu et al., 2024). The approach uses a parameter called genomic effective daughter contribution (EDC) gain (φ_c) for genotyped animals and the propagated EDC (φ_i^{propg}) for non-genotyped animals, to combine the genomic reliability gain with the conventional EBV reliability to obtain the final GEBV reliability.

Step 1: Calculate the genomic EDC gain (φ_c)

This step comprises five sub-steps:

1) compute the DGV reliabilities for all the genotyped animals were computed using the model (1).

2) compute theoretical gain in genomic EDC as:

$$\varphi_i = \frac{1-h^2}{h^2}\left(\frac{r_{DGV}^2}{1-r_{DGV}^2} - \frac{r_{EBV}^2}{1-r_{EBV}^2}\right) \tag{6}$$

we denoted the mean of φ_i as $\bar{\varphi}$.

3) compute φ_i^{propg} using $\bar{\varphi}$ as input to propagate the genomic information from genotyped animals to their non-genotyped relatives via pedigree (VanRaden and Wiggans, 1991, Liu et al., 2004).

4) compute the combined total theoretical EDC.

For genotyped animals:

$$\varphi_i^{total} = \varphi_i^{conv} + \varphi_i \tag{7}$$

For non-genotyped animals:

$$\varphi_i^{total} = \varphi_i^{conv} + \varphi_i^{propg} \tag{8}$$

5) convert to the final theoretical GEBV reliability:

$$R_i^2 = \frac{\varphi_i^{total}}{\varphi_i^{total} + \frac{1-h^2}{h^2}} \tag{9}$$

Note that sub-steps 1 through 5 must be applied to both the full and reduced datasets.

6) compute an adjustment factor (f) based on the validation bulls:

$$f = \frac{E(\varphi_E)}{\bar{\varphi}_E} \tag{10}$$

where $E(\varphi_E)$ is the expected EDC value:

$$E(\varphi_E) = \frac{1-h^2}{h^2} \times \frac{E(R_E^2)}{1-E(R_E^2)} \tag{11}$$

where

$$E(R_E^2) = \overline{R_L^2} - E(\Delta R^2) \tag{12}$$

where $\overline{R_L^2}$ is the mean reliability of GEBV of the validation bulls from the full dataset, $E(\Delta R^2)$ is the expected change in reliability of GEBV:

$$E(\Delta R^2) = var(\hat{u}_L - \hat{u}_E)/\sigma_u^2 \tag{13}$$

where $\hat{u}_L$ and $\hat{u}_E$ are the GEBV of the validation bulls from the evaluation using the full and reduced datasets, respectively; σ_u^2 is the additive genetic variance. $\bar{\varphi}_E$ is the theoretical EDC value of the validation bulls from the reduced dataset:

$$\bar{\varphi}_E = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{1-h^2}{h^2} \times \frac{R_{E_i}^2}{1-R_{E_i}^2}\right) \tag{14}$$

7) compute the adjusted genomic EDC gain (φ_i^{adj}) for all the genotyped animals with the f factor derived from equation (10):

$$\varphi_i^{adj} = \frac{1-h^2}{h^2}\left(\frac{r_{DGV}^2}{1-r_{DGV}^2} \times f - \frac{r_{EBV}^2}{1-r_{EBV}^2}\right) \tag{15}$$

The constant parameter of φ_c is the mean of the adjusted genomic EDC gain (φ_i^{adj}):

$$\varphi_c = \frac{1}{n}\sum_{i=1}^{n}\varphi_i^{adj} \tag{16}$$

Step 2: Propagate genomic information

This step is the same as sub-step 3) above to obtain φ_i^{propg} for the non-genotyped animals but using φ_c as the input data.

Step 3: Compute the final reliability of GEBV for all animals

For genotyped animals:

$$\varphi_i^{total} = \varphi_i^{conv} + \varphi_c \tag{17}$$

For non-genotyped animals, use the equation (8). The final reliability of GEBV for all the animals can be calculated via equation (9).

Data

To evaluate the approaches, a dataset comprising 47,124 Finnish Red dairy cows with 305-day milk yield records from first lactation was used. The analyses included 19,757 genotyped animals with 46,914 SNPs, and the pedigree encompassed 64,808 animals. The heritability of the trait was set to 0.44, and the proportion of RPG effects was assumed to be 0.30.

Results & Discussion

Reliabilities of the genotyped animals

The mean (SD) reliability of GEBV were 0.66 (0.09), 0.66 (0.09), 0.57 (0.10) from the exact, Luke, and Interbull approach for the genotyped animals, respectively. Figure 1 shows the GEBV reliabilities from the exact method versus those from the Luke method (left panel) and the Interbull method (right panel). Overall, the correlations between Luke/Interbull and exact method were close to one.

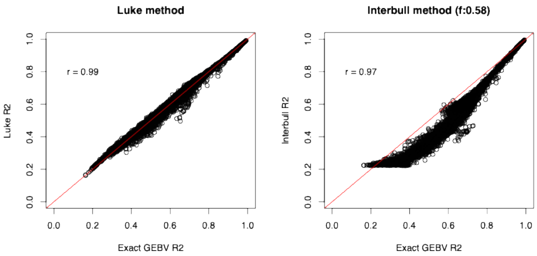

Figure 1. Scatter plot and Pearson's correlation coefficients (r) of the reliabilities of genomic estimated breeding values (GEBV) for genotyped animals via the Luke method (y-axis) versus the exact method (x-axis) (left panel) and via the Interbull method (y-axis) versus the exact method (x-axis) (right panel). The solid red line acts as a reference line with intercept 0 and slope 1

Reliabilities of the non-genotyped animals

The mean (SD) GEBV reliabilities for non-genotyped animals were 0.48 (0.17), 0.44 (0.15), and 0.43 (0.17) using the exact, Luke, and Interbull approach, respectively. Figure 2 presents the GEBV reliabilities from the exact method against those from the Luke approach (left panel) and the Interbull approach (right panel). While the correlations between the Luke/Interbull and exact approaches were slightly lower than those observed for genotyped animals, they remained high overall.

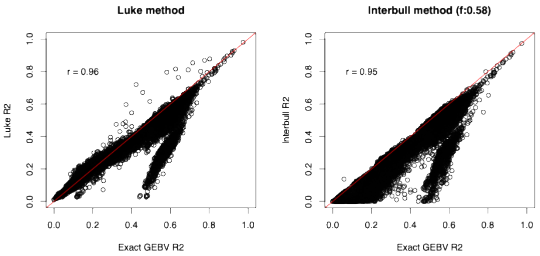

Figure 2. Scatter plot and Pearson's correlation coefficients (r) of the reliabilities of genomic estimated breeding values (GEBV) for non-genotyped animals via the Luke method (y-axis) versus the exact method (x-axis) (left panel) and via the Interbull method (y-axis) versus the exact method (x-axis) (right panel). The solid red line acts as a reference line with intercept 0 and slope 1

In this study, we compared the Luke and the Interbull approaches for approximating GEBV reliabilities. Both approaches computed GEBV reliabilities separately for genotyped and non-genotyped animals and required conventional EBV reliabilities for all animals in the pedigree.

The Luke approach used the information from a PBLUP model to derive ERC which served as weights in a SNPBLUP model that incorporates genomic information when computing GEBV reliabilities for genotyped animals. Similarly, for non-genotyped animals, the genomic information was included indirectly by applying additional weights derived from the genotyped animals within a weighted PBLUP model. An important feature of this approach is that the models for computing GEBV reliabilities include only a general mean and genetic effects, while the weighting scheme and relationship structure differ between genotyped and non-genotyped groups.

The Interbull approach employed a constant parameter (φ_c) derived from the Interbull GEBV test, to simplify computations in large-scale genotyped populations. The φ_c was propagated to the non-genotyped relatives via pedigree to obtain their respective propagated EDC gain (φ_i^{propg}). The final EDC values were then calculated by combining the conventional EDC with φ_c for genotyped animals and φ_i^{propg} for non-genotyped animals. GEBV reliabilities were subsequently derived from the total EDC using equation (9).

The results showed that the approximated GEBV reliabilities from both approaches were in close agreement with the exact values, supporting their applicability in practical genetic evaluations.

It is important to note that a key feature of the Interbull approach is the derivation and use of the genomic EDC gain parameter (φ_c), which can be repeatedly applied to approximate GEBV reliabilities. However, because φ_c is directly linked to the Interbull GEBV Test, it must be re-estimated and updated each time a new GEBV test is conducted. This feature offers the computational simplicity and efficiency. In contrast, the Luke approach requires precise calculation of ERC weights for each computation of reliabilities, which may increase computational demands.

The RPG effects need to be considered to avoid overestimating the reliability of GEBV; however, these effects were not explicitly incorporated in either approach. The Luke approach employed a blended method to approximate GEBV reliabilities for genotyped animals, thereby retaining the primary advantage of the SNPBLUP model, that is, even as the number of genotyped animals increases, the dimensionality of the coefficient matrix of the MME remains fixed, depending solely on the number of SNPs. The Interbull approach implemented an adjustment factor (*f*) to scale down the theoretical GEBV reliabilities to ensure an appropriate genomic reliability level for young selection candidates.

This study used a relatively small dataset to ensure the feasibility of computing the exact GEBV reliabilities by directly inverting the coefficient matrix of the MME. However, routine single-step genomic evaluations in practice often involve millions of genotyped animals, thus, a larger and more representative datasets might be more appropriate to further evaluate these approaches.

Conclusions

This study compared two approaches for approximating genomic reliabilities for both genotyped and non-genotyped animals. The results demonstrated that both approaches produced reliability estimates in close agreement with the exact reliabilities computed using the full dataset in a ssGBLUP evaluation. Importantly, both methods indirectly accounted for residual polygenic (RPG) effects without explicitly including them in the model. Although the Interbull method relies on the Interbull GEBV test, both approaches offer effective strategies for obtaining GEBV reliabilities in practical large-scale single-step evaluations.

Acknowledgments

The authors are grateful to Viking Genetics (Randers, Denmark) and Nordic Cattle Genetic Evaluation (Aarhus, Denmark) for providing the data.

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Is ‘Farmeromics’ the missing omic?

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Abstract

Many publications requiring access to large datasets from commercial conditions, such as dairy genetics, note challenges with access to and quantity of data. Challenges include insufficient records and inconsistent trait definitions. This could be improved through developing closer relationships between farmers and researchers. In the same way that scientists innovate to develop the fields of genomics, phenomics, metabolomics, etc., can we advance our research by developing ‘farmeromics’ – defined as the study of farmer-driven, biological data recording, at scale? The purpose of this investigation was to compile examples of research studies that involved farmers in their co-design, including the calf vitality project, Feeding the Genes and ImProving Herds These examples could spark discussion on ways to strengthen collaboration between farmers, scientists and stakeholders to reach shared data-gathering objectives.

Key words: Co-design, farmer engagement, phenotyping, farmeromics

Introduction

It is likely that a reader of this Bulletin will intuitively recognize the importance of phenotypic data to drive genetic gain in dairy cattle and will easily recall Professor Mike Coffey’s famous phrase, ‘in the age of the genotype, phenotype is king’ (Coffey, 2020). Yet, data availability remains a stumbling block in many research activities. Once example of this is calf health. For instance, in 2022, 1 in 5 Australian dairy herds systematically recorded calving traits (Axford et al., 2023). This low participation rate is inconsistent with trait preference data suggesting that farmers value calving ease similarly to mastitis, type traits and temperament - traits that are so essential that they are often included in national breeding indices (Axford et al., 2025a). Similarly, in Canada, up to 15% of farms had accessible calf health records (Hyland, 2022) suggesting that the problem isn’t isolated to a particular country.

It’s no surprise, then, that publications on the genetics of dairy health traits often include commentary on data-related challenges. Authors frequently cite under-reporting, inconsistent trait definitions, and a lack of standardization (Cuttance & Laven, 2019; Lombard et al., 2019; Lynch et al., 2024). Additional concerns include non-digitised storage and inaccessible data sources (Edwards et al., 2024). These issues are so widespread that they are frequently cited, yet solutions remain elusive. Proposing meaningful solutions remains far more difficult.

This raises an important question. What role can researchers play in getting closer to the source of the data - the farm and the farmer? Just as scientists have pioneered the fields of genomics, phenomics, metabolomics, etc., can we advance our research by developing ‘farmeromics’ – the study of farmer-driven, biological data recording, at scale? Encouragingly, both farmers and scientists have a strong history of driving change. Involving farmers more directly in research may unlock innovation that leads to more effective, practical improvements in research practice.

This investigation aims to compile examples of studies that involved farmers in their co-design and to highlight the impact that emerged from these collaborations.

Materials and Methods

Defining Farmeromics?

As introduced earlier, ‘farmeromics’ refers to the study of farmer-driven, biological data recording, at scale. To clarify this concept, it can be broken down into three components.

1. **Farmer driven.** This aspect focuses on listening to farmers to understand the motivators for data collection. Key questions include:
   - What problems do farmers want to solve using data?
   - What motivates farmers to record data in the first place?
2. **Biological data**. This refers to the type of data that is relevant to both farmers and researchers. It prompts consideration of:
   - What data do researchers need to answer scientific questions?
   - What data might already be available on farms?
3. **Recording at scale.** This component addresses the practicality of large-scale data collection efforts. It asks:
   - Do current recording practices align with the standard operating procedures common on today’s farms?
   - Have researchers actively sought out this data from farmers?

The Approach

Retrospectively, a selection of successful projects that incorporated elements of “farmeromics” was compiled. These examples are not intended to be comprehensive, but rather illustrative and are offered to spark conversation. Each case demonstrates how farmer involvement in data collection and research design contributed, in part, to meaningful outcomes.

Results & Discussion

In Table 1, we introduce 3 research activities that featured a close association with farmers that are discussed in this paper.

Table 1. Project overview

Project	Aim
Calf vitality	Estimate variance components for calf health traits
Feeding the Genes	Study genetic by environment interaction in herds that varied by feeding system
ImProving Herds	Compare the cows’ contribution to profit between cows differentiated by national index rank.

Example 1: Calf vitality

Australia’s Calf Vitality Project aimed to estimate variance components for calf health traits in a country without obligatory or habitual recording practices (Axford et al, 2025b). During the initial stages of the project when farmer recruitment was underway to build a bespoke dataset, farmers proposed an additional phenotype which was a subjective score. In their words, they wanted to record calves that were ‘rippers’ or ‘duds’. These colloquial terms were formed into a subjective scoring tool trait with 5 levels where A was a vigorous calf (a ‘ripper’), B was a good calf, C was an average ‘ok’ calf, D was a dull calf that lacked vigour (a ‘dud’) and E was a dead calf. The approach was modelled on the familiar system for recording workability traits (milking speed, temperament and likeability) that was initiated thirty years ago and still well used today (Beard, 1993). Images reflecting the scoring tool were developed, as shown in Figure 1, to introduce the idea to project participants.

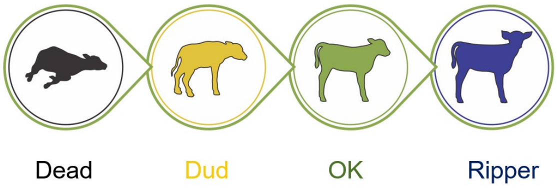

Figure 1. Visual descriptions of the calf vitality scores

What was the outcome?
Over 50 farmers participated in the calf vitality project and contributed detailed health phenotypes and genotypes from ~20,000 calves. While it is difficult to apportion the success of the data collection activity to one or more factors, it is likely influenced by the high level of farmer interaction during the initiation of data collection. At the completion of data collection, about half of the participating farmers actively recorded this new trait. Of all the calf traits, calf vitality had the highest estimated heritability (11%) as described, along with more detailed genetic parameters in Axford et al. (2025b). Further, this trait attracted farmer engagement to the project and generated the most conversation of all the calf traits during industry events.

Why did it work?
Underpinning the definition of 'farmeromics' is the principle of co-design. In this context, co-design' means involving farmers and advisors in a project from the beginning to increase engagement, acceptance, transparency and reduce the possibility of failure. Fleming et al. (2023) extends co-design to include 'co-development' and 'co-delivery' as part of a Co-3D spectrum for project delivery. Calf vitality was 'co-designed' by first listening to the problems as farmers expressed them. Then, we developed a mechanism of recording data that fitted with their routines and targeted a problem that farmers wanted to solve. When the value proposition was strong (ie, breeding for healthier calves), and the barriers to participation were low (ie, simple recording), farmers were willing to provide data that was earlier though to be unavailable.

Example 2: Feeding the Genes
With hindsight, 'Feeding the Genes' (Morton et al., 2015) was successful, in part due to elements of 'Farmeromics'. The aim of this project was to investigate the interaction between genetic merit and feeding system on the performance of cows in Australia herds. A feature of the Australian dairying system is a heterogeneity of feeding systems. Dairy Australia has identified five broadly defined feeding systems ranging from predominantly pasture and conserved fodder with low concentrate use, through to total mixed rations. Farmers were asking questions about the performance of high genetic merit animals in each of these systems, especially for phenotypic measures of milk production and longevity.

This study required feeding system data that was not routinely recorded and stored for herd-recorded herds. A survey was conducted to gather the required data and it is here that that we find principles of 'Farmeromics'. The survey was:

- focused on questions that farmers were asking,
- short – with just 5 questions,
- targeted – candidate herds with selected on the basis of production and longevity data, and
- backed by industry advocates – a multi-disciplinary team that included well-known spokespeople were leading the survey.

What was the outcome?
The survey attracted a high response rate of 24% meaning that ~300,000 lactations from 505 herds were able to be used in the milk production analysis. The ability to link feeding systems and herd performance enabled research that concluded there were clear benefits to using high genetic merit sires in each of the five feeding systems.

Why did it work?

In this example, the availability of feeding system data was critical to the research but not readily available. By asking farmers for this information in a way that was simple and with a clear value proposition, the response rate exceeded expectations. 'Simple' for the end user shouldn't be confused as 'easy' for the researcher. Significant effort was required to develop the survey questions with a multi-disciplinary team of advocates in order to achieve success.

Example 3: ImProving Herds

Many models that are used to derive economic indexes target an outcome based on a definition of profit, for example Pro$ in Canada (Van Doormaal et al., 2015), Balanced Performance Index (BPI) in Australia (Byrne et al., 2016), Net Merit in the United States of America (Van Raden et al., 2025) and others. Farmers and advisors often seek information that validates the profit predicted in indices with practical, 'real' herd examples. One of the aims of Australia's ImProving Herds project was to compare the cows' contribution to profit between cows differentiated by BPI rank. This required a comparison of individual cow lifetime performance information with farm financial data to calculate margin over feed and herd costs (MOFH) as a measure of contribution to farm profit (Newton et al., 2017). However, detailed farm financial records are rarely captured in routine herd recording. In this project, two disparate datasets were initially combined from different agencies, with agreement from farmers. Later, consultants with specific expertise in compiling and assessing farm financial data were engaged to collect this data for a diverse range of herds with high value herd performance and genomic data that were important to the study. This information enabled an analysis that linked farm financial performance with genetic merit at an individual cow level resulting in the calculation of each cow's contribution to profit. This formed the basis of a series of practical case studies that appealed to farmers and service providers.

What was the outcome?

On average, high-BPI cows contributed ~AU $300 per cow per year more to margin-over-feed and herd costs (MOFH) than did their low-BPI herd mates (Newton et al., 2021). Additional milk income easily compensated for the higher feed costs associated with high BPI cows. Further, a sensitivity analysis showed that this result holds true even if milk price fell by 50% while feed cost stayed the same or feed cost doubled and milk price stayed the same. As the case studies were conducted in a range of environments, this project generated many stories that formed a well-used extension resource.

Why did it work?

In this project, looking beyond the traditional data sources revealed opportunities previously thought 'too hard'. As discussed by Newton et al., (2021), iterative discussions with dairy farmers, economists, service providers and technical independent geneticists from overseas were required to develop this research study. Consistent with co-design principles, communication and early extension activities were incorporated within a research project and this enabled the involvement of representatives from across the herd improvement industry throughout the project. While challenging to manage, the iterative feedback cycles on the project methodology and messaging created new opportunities.

Conclusions

In these three examples, involving farmers

- revealed new data that was not previously unknown to exist, through discussion,
- fine-tuned the research question, through conversations, so that the project's discoveries were more meaningful to the intended audience,
- ensured that proposed practice changes had considered practical implications, and,

- benefited communication and extension activities that raised awareness of the research findings.

At its heart, 'farmeromics' is a prompt to ask, 'how can we do a better job of working with farmers to capture important data and make great research projects even more meaningful?'

Acknowledgments

The calf vitality project was funded by DairyBio, an initiative of Dairy Australia, Agriculture Victoria and The Gardiner Foundation and contributed to a PhD project at La Trobe University. The author thanks her PhD supervisors, DataGene colleagues, Kevin Jones, Nancy Charlton and calf vitality participants for the conversations that sparked this article.

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Integration of MACE breeding values into Swiss multi-trait test-day model evaluation

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Abstract

In this study, we integrated Multiple Across Country Evaluation (MACE) information for Interbull (ITB) bulls into the Swiss Test-day model (TDM). The 9-trait TDM includes test-day records of milk, protein and fat from the first three lactations, while total yield indices submitted to ITB are averages of 305d yields for lactations. A bull was considered to have relevant MACE information if its reliabilities for all indices in MACE were at least 0.1 units higher than its reliabilities from the Swiss TDM. With this integration, the Swiss TDM gained information for round 5,800 bulls with MACE index reliabilities exceeding 0.5.

The integration process had three steps. 1) For selected bulls, the multitrait reversed reliability approximation was used to estimate effective record contributions (ERC) for Swiss and MACE yield indices, based on their respective reliabilities. 2) Yield indices and ERCs were used to calculate multitrait deregressed proofs (DRP) separately for Swiss and MACE evaluation. Correlations between the evaluated indices and pedigree relationships were accounted during the ERC and DRP calculations. 3) Based on the DRPs and ERCs for domestic and MACE indices, pseudo-observations approximating the additional information in the MACE evaluation were calculated for the selected bulls. As a result, for each selected bull a DRP and ERC for milk, protein, and fat were obtained.

The original Swiss TDM describes breeding values using 45 random regression coefficients. The DRP was included in the model as a separate trait, weighted by its ERC. The genetic correlation between pseudo trait and lactation averages of the original traits was assumed to be 1. MACE inclusion improved correlations between MACE and Swiss indices to 0.99 (from 0.78–0.80 for milk, fat, protein). This demonstrates a good alignment between the two evaluation systems. Integration of MACE is now implemented successfully in the Swiss single-step routine genetic evaluation.

Key words: Test-day model, MACE integration, three-step approach, production

Introduction

Accurate genetic evaluation is essential for accelerating genetic improvement in dairy cattle breeding programs (Schaeffer, 1994). Integrating multiple across-country evaluation (MACE) proofs into single-step genomic analyses enables the inclusion of reliable international information, particularly for foreign progeny-tested sires with no or only few domestic offsprings. This integration improves the accuracy of estimated breeding values (EBV), enhances the genetic connectedness between countries, and supports more robust selection decisions in an increasingly globalized dairy industry (Sullivan, et al. 1999, Boerner et al., 2022).

Recent research has proposed several strategies to incorporate MACE information into single-step evaluations. For instance, Nieuwhof et al. (2023) developed a method using deregressed proofs (DRPs) that account for relationships among MACE bulls, improving reliability and reducing bias compared to approaches that assume unrelatedness of bulls. Similarly, Bayesian methods such as ssGBayes and trait-specific deregression techniques have shown promising results in Canadian and Walloon Holstein populations (Strandén et al., 2022; Splichal et al., 2023)

While some approaches simplify the relationship between international and domestic genetic effects, e.g., by treating MACE DRPs as auxiliary traits or integrating them into reduced-rank test-day models, these often compromise consistency with the full model structure. In contrast, the approach presented here integrates MACE-derived pseudo-observations directly into the full Swiss multi-trait test-day model (TDM). These pseudo-observations are treated as weighted, trait-specific contributions aligned with the genetic lactation curves, ensuring consistency with the model's structure and preserving trait definitions across data sources.

This study describes a three-step approach to integrating MACE information into the Swiss TDM and demonstrates its validity through comparisons of EBV and reliabilities from pedigree-based BLUP (PBLUP) and single-step GBLUP (ssGBLUP) before and after blending. Furthermore, it evaluates the impact of genomic information on the blending procedure, particularly for genotyped bulls. The implementation is now part of the Swiss routine single-step evaluation pipeline.

Materials and Methods

Data

The raw phenotypic dataset encompassed 49,744,608 test day records for the yield traits: milk, fat and protein each in kg for days in milk (DIM) between 5 and 365. Different milk testing methods (A4, ATM4 and AT4) were used to record the data on 1,753,643 cows born between 1984 and 2023.

The total number of herds was 34,896. The number of herd-test-day-parity (HTD) classes was 4,437,539 and the number of time-region-age-parity-season (TRAPS) classes was 476. Time was divided into half-year groups based on the test day, starting from year 2000. Region was defined via geographic classification. Age was divided into monthly classes (≤19 month, 20-24 month, 25-28 month and ≥ 28 month). Parity was divided into first, second and third and ongoing. Season was divided in January-March, April-June, July-September, October-December.

Genotypes of 153,499 animals were included in the single-step evaluations. As animals were genotyped with different SNP panels, all genotypes were imputed together (one reference panel) to 125K SNP following the routine imputation process at Qualitas with FImpute (v3.0; Sargolzaei et al., 2014).

The pedigree was built up using cows with phenotypes as well as young, genotyped animals and pruned to three generations and finally included 2,367,788 animals. Genetic groups were divided by breed but also separated over different periods of time and sex.

Swiss test-day model

A multi-trait (yield traits), multi-parity (5 lactations) random regression model, defined as

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Z_p p} + \boldsymbol{Z_a a} + \boldsymbol{\epsilon}, \qquad (1)$$

was used, where $\boldsymbol{y}$ is the vector of observations, $\boldsymbol{\beta}$ represents the fixed effects of HTD and the fixed lactation curve for TRAPS, $\boldsymbol{p}$ is the vector of random permanent environmental effects, $\boldsymbol{a}$ is the vector of random genetic effects, and $\boldsymbol{\epsilon}$ represents the random residuals. $\boldsymbol{X}$ and $\boldsymbol{Z_p}, \boldsymbol{Z_a}$ are respective incidence matrices.

To account for the accuracy of the phenotype, different weights were used for different milk testing method (1=A4, 0.94=ATM4, 0.88=AT4).

The TRAPS effect was modeled using a six-order Legendre polynomial. Both the genetic and permanent environmental lactation curves were modeled using fourth-order Legendre polynomials. Lactations 4 and 5 were treated as repeated measures of the third lactation for the fixed effects and the genetic effect, while lactation-specific effects were included for permanent environmental effects. Assumptions were that

$$\boldsymbol{var}\begin{bmatrix}\boldsymbol{a}\\\boldsymbol{p}\\\boldsymbol{\epsilon}\end{bmatrix}=\begin{pmatrix}\boldsymbol{G_O}\otimes\boldsymbol{A} & \boldsymbol{0} & \boldsymbol{0}\\\boldsymbol{0} & \boldsymbol{I}\otimes\boldsymbol{P} & \boldsymbol{0}\\\boldsymbol{0} & \boldsymbol{0} & \boldsymbol{R}\end{pmatrix},\quad(2)$$

where $\boldsymbol{G_O}$ is the covariance (45×45) matrix for the random genetic effects, assumed to be the same for each cow. $\boldsymbol{A}$ is the pedigree relationship matrix between the animals used for pedigree BLUP (PBLUP). To include genomic information, the **A** matrix was replaced by an augmented matrix (**H**) that includes both pedigree and genomic information, and was incorporated by applying ssGTABLUP (Mäntysaari et al., 2017), where the genomic relationship matrix (**G**) was constructed using VanRaden method I (VanRaden, 2008) and blending the **G** matrix with a 5% residual polygenic component. Pedigree inbreeding coefficients were incorporated into both $\mathbf{A}^{-1}$ and $\mathbf{A}_{22}^{-1}$. Genetic groups were accounted for in the single-step models through a partial QP transformation that excluded $\mathbf{G}^{-1}$ from the QP matrix (Koivula et al., 2021).

$\boldsymbol{P}$ is the covariance (75×75) matrix for the permanent environmental effects.

$\boldsymbol{R}$ is the covariance matrix of the residuals, composed of 3×3 covariance matrices corresponding to four lactation periods based on DIM: 5–45, 46–115, 116–265, and 266–365. Each period was associated with its own 3×3 residual covariance matrix.

Lactation specific breeding values were calculated by summing up the breeding values for DIM 5 to 305. Combined breeding values were calculated as a weighted sum of lactation specific breeding values by using weight of 1/3 for each lactation. The combined breeding value was standardized by subtracting the mean EBV of cows aged between 6 and 8 years. Standardized breeding values for milk, protein and fat and their reliabilities were submitted to Interbull for all bulls.

Bulls Chosen to be Blended

After performing MACE, ITB returned MACE breeding values and reliabilities. Bulls were selected for blending if their MACE reliability exceeded 0.5 and exceeded their domestic reliability by more than 0.1 units, irrespective of whether the bull was genotyped or not.

In total 5,864 bulls were selected per yield trait, whereof 5,466 were genotyped and 247 had information in domestic evaluation.

Calculation of pseudo-observations

Integration of additional information in MACE breeding values for milk, protein and fat to domestic evaluation was done using deregressed proofs (DRP) as pseudo-observations and effective record contribution (ERC) as weights. The integration process includes calculating ERCs from reliabilities and DRPs from EBV based on domestic and MACE proofs (Pitkänen et. all 2020, Pabiou et. all 2018, Vandenplas et. all 2014).

DRPs and ERCs were calculated assuming that EBV and reliabilities are from linear multitrait animal model:

$$\begin{bmatrix}y_m\\y_p\\y_f\end{bmatrix}=\mu+a+e,\qquad(3)$$

where y_m, y_p and y_f are combined 305d observations for milk, protein and fat, μ is intercept, a is random genetic effect, containing breeding values for combined milk, protein, and fat for each animal, and e is the residual effect. The variance components for a and e were derived for 305d yields based on test-day model variance components. Residual variance covariance matrix included variation due to residual and permanent environment effects in the test-day model.

In the first step, two sets of reliabilities—one from the domestic evaluation and one from MACE—were used to calculate effective record contributions (ERC_D and ERC_M) for combined milk, protein, and fat.

In the second step, DRPs for combined milk, protein and fat for domestic (DRP_D) and MACE (DRP_M) were calculated based on combined EBV from evaluations and using ERCs from the first step as weights. The standardized EBV were back transformed to original scale before calculations.

Since DRP_M, contains information also from domestic animals, it can't be directly included in the model due to double counting of information. In the third step, the double counting was removed by calculating DRP^B, ERC^B, within trait as:

$$ERC^B = ERC_M - ERC_D \quad (4)$$

$$DRP^B = \frac{DRP_M \cdot ERC_M - DRP_D \cdot ERC_D}{ERC^B}. \quad (5)$$

Blending Model

Pseudo-observations for milk, protein and fat yield were included as separate traits for the test-day model assuming the pseudo-observation is a weighted sum of 305d breeding values of lactations 1 to 3. The model for pseudo-observations for animal *i* is:

$$\begin{bmatrix} DRP_{im}^B \\ DRP_{ip}^B \\ DRP_{if}^B \end{bmatrix} = \mu + \boldsymbol{C}\boldsymbol{a}_{i1} + \boldsymbol{C}\boldsymbol{a}_{i2} + \boldsymbol{C}\boldsymbol{a}_{i3} + \boldsymbol{e}_i,$$

where

$$\boldsymbol{C} = \boldsymbol{I}_3 \otimes \boldsymbol{C}_{305},$$

and

$$\boldsymbol{a}_{il} = \begin{bmatrix} a_{i,ml} \\ a_{i,pl} \\ a_{i,fl} \end{bmatrix}.$$

The vector $\boldsymbol{C}_{305}$ is sum of covariable values for genetic lactation curve between DIM 5 to 305. Residual covariance matrix for pseudo-observations was the same as used in model (3). The genetic regression coefficients, $a_{i,tl}$, for trait *t*, and lactation *l*, are the same as for the test-day observations. Hence, the genetic correlation between MACE and domestic evaluation was assumed to be 1. All calculations were done using MiX99 software suite, Release X/2023.

Results & Discussion

In the following only the results for milk are shown (Figure 1, 2 and 3) and discussed, because they are similar for the other traits.

PBLUP reliabilities

Integrating MACE proofs improved correlations between MACE and domestic reliabilities (R2) towards the expected value of 1 (Figure 1). The intercept of the reliabilities decreased, and the slope increased indicating that the reliabilities after blending are not biased.

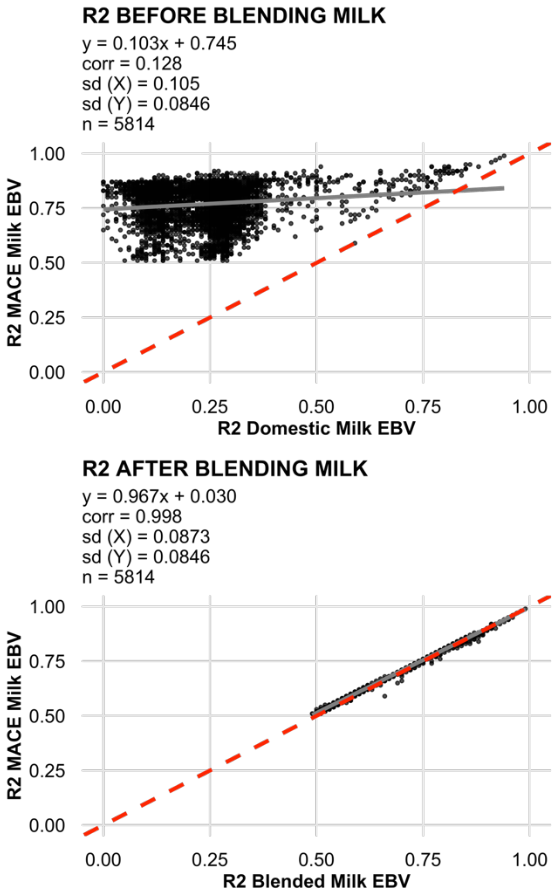

Figure 1. Comparison plots between PBLUP reliabilities (R2) before (top) and after blending (bottom) with MACE reliabilities. The red, dotted line represents the expectation if blending works.

PBLUP breeding values

Integrating MACE proofs improved correlations between MACE and domestic EBV (Figure 2). The intercept of the EBV increased, while the slope of the EBV decreased.

The intercept deviates from 0. However, compared to the scale of the EBV ranging from -2000 to +2000 this deviation is small. More important is the slope which is quite close to the expectation. Traits are modelled independently in MACE but dependently in the domestic ERC and DRP calculation, which might explain the deviation from the expectation.

Overall, the results are in accordance Pitkänen et al. (2019 and 2020), where similar blending strategies were applied to Nordic Holstein evaluations, and Vanderick et al. (2025). In contrast to this study, their approach sets the residual correlation for DRP computation to zero.

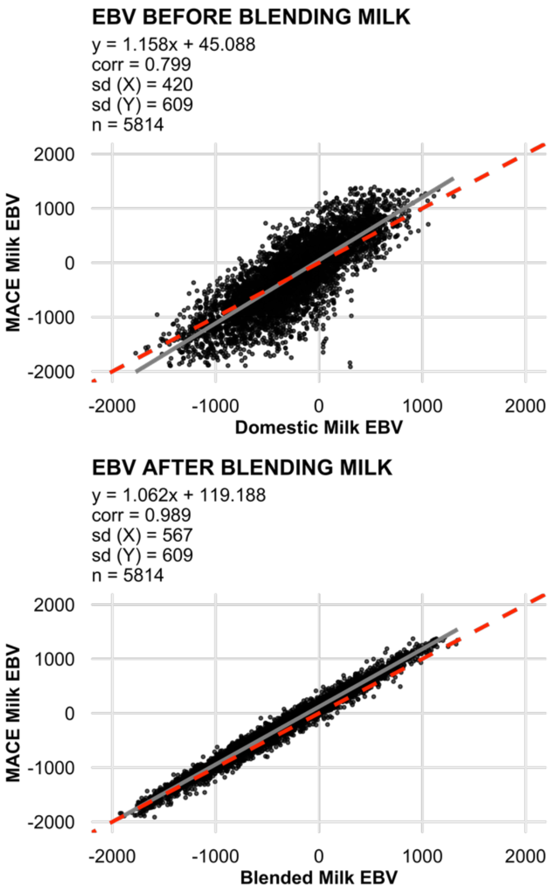

Figure 2. Comparison plots between PBLUP breeding values (EBV) before (top) and after blending (bottom) with MACE EBV. The red, dotted line represents the expectation if blending works.

ssGBLUP reliabilities and breeding values

The integration of genomic information led to a higher standard deviation of the ssGBLUP reliabilities compared to their MACE equivalent (Figure 3). All genotyped bulls gained in reliability. The reliability of non-genotyped bulls has not changed after blending.

The standard deviation of the EBV increased when integrating genomic information.

These findings are consistent with the observations of Rostellato et al. (2024) who demonstrated that genomic-free Single-Step EBVs used for MACE derivation increase reliability, particularly for genotyped animals.

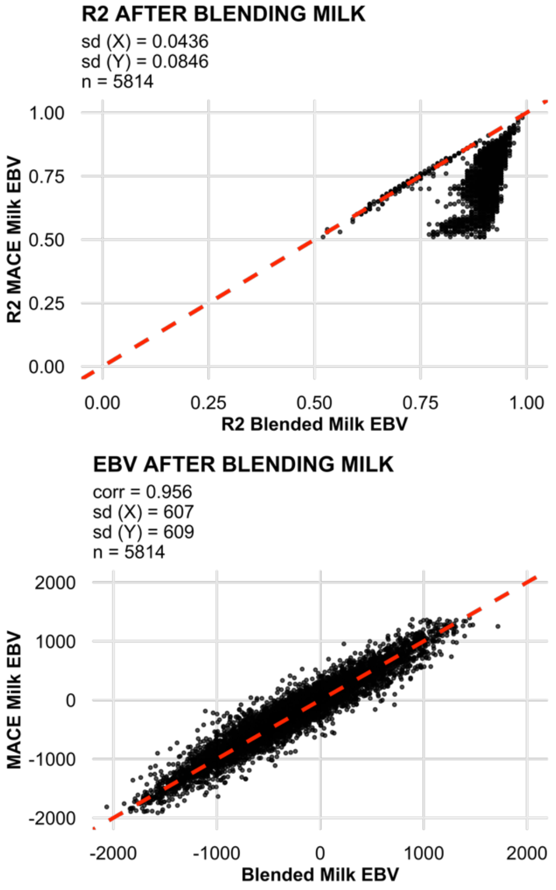

Figure 3. Comparison plots between ssGBLUP – and MACE reliabilities (R2) on top and ssGBLUP – and MACE breeding values (EBV) on bottom. The red, dotted line represents the expectation for PBLUP.

Conclusions

The three-step approach integrates well MACE results into PBLUP and ssGBLUP and allows recovering indirectly a large amount of phenotypic information. All available external sources of information are correctly propagated avoiding double counting of contributions due to relationships and due to own records. Furthermore, the results are in accordance with the findings from the literature. Therefore, the approach proves to be a good choice for the Swiss genomic evaluation system integrating domestic and MACE EBV and is now implemented successfully in the routine genetic evaluation.

Acknowledgments

Braunvieh Switzerland is acknowledged for providing the data used in this study.

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Genetic and genomic relationships among Canadian Holstein dairy cattle population and international Holstein bulls

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Abstract

The increasing level of inbreeding in dairy cattle populations can be a concern for researchers, producers, and artificial insemination (AI) companies. High inbreeding levels can lead to the accumulation of deleterious recessive variants, depression of the mean value of economically important traits, and a reduction in available genetic diversity in the population. Advancements in reproductive technologies and the integration of genomic information into genetic evaluations have contributed to rising inbreeding levels. Given the global interest in inbreeding, there is a need to monitor inbreeding trends and develop strategies to manage its adverse effects while ensuring continued genetic progress. The objective of this study was to measure the current trends in genetic and genomic relationships among Canadian Holstein cows and international Holstein bulls. Pedigree and genotype data were provided by Lactanet Canada (Guelph, ON). Genotype information was available for 168,995 animals, for which a pedigree of 616,258 animals was extracted. Among genotyped animals, 8,491 bulls were born between 2000 and 2023, and 131,139 cows were born between 2010 and 2024. The average pedigree completeness index of all genotyped animals was greater than 99%, with a maximum pedigree depth of 30 generations. Genetic relationship values were estimated using pedigree data (R-value) and genomic data (GR-value). R-value and GR-value represent the expected and realized percentage of shared DNA between an animal and a defined reference population, respectively. This was done by iteratively tracing back the gene contribution of an animal to the reference population. In this study, the reference population was defined as currently active cows and heifers enrolled in milk recording without a documented left-herd date on the latest test day in April 2024. Results show a yearly increase in relationship within the reference and bull populations. The average genetic relationship between bulls and the reference population ranged from 9.3% to 26.5% (R-value) and from 12.9% to 40.8% (GR-value). Among bulls, those with United States registration codes had the highest relationship value with active Canadian cows and heifers, with R-value and GR-value estimates of 20.8% and 30.4%, respectively. Conversely, bulls registered in the Czech Republic had the lowest average relationship values, with R-value and GR-value estimates of 17.1% and 24.3%, respectively. Selecting sires with low average relationship values among defined reference populations as a mating strategy could reduce or maintain inbreeding at acceptable levels while preserving genetic diversity.

Key words: Genetic relationship, inbreeding diversity, Holstein, dairy cattle

Introduction

The availability of genotype information has driven rapid advancements in breeding programs across most intensive dairy producing countries, particularly following the implementation of genomic selection (García-Ruiz et al., 2016; Miglior et al., 2017). Moreover, the international exchange of genotypes among partner countries has been reported to produce higher gains in reliability estimates of breeding values for economically important traits (Schenkel et al., 2009). These exchanges contribute not only to the increase of genetic progress, but also to greater genetic relatedness of animals across and within countries. This increased genetic connectedness leads to rising levels of inbreeding within a given population.

Increased inbreeding can reduce genetic variation, which may limit the response to selection, lower the mean value of economically important traits, and promote the accumulation of deleterious recessive alleles. Ultimately, this can increase the frequency of genetic defects and result in substantial economic losses for producers. Therefore, monitoring relatedness and inbreeding levels within a population is important for sustainable genetic progress. One approach to monitoring genetic relatedness within a population is by estimating genetic relationship values between actively producing cows and currently available sires. Genetic relationship values measure the proportion of DNA an animal shares with a predefined reference population and can be estimated using either pedigree information (R-values) or genomic data (GR-values).

As pedigree and genotype information accumulates, genetic relationships among animals in a population can be more accurately estimated. These relationships depend on both the number of descendants an animal contributes to the active population and the number of ancestors that have many descendants in the active population. Consequently, genetic relationships are bound to change continuously as actively producing animals are culled or lost from the population. Increased availability and use of young genomic sires can reduce the heavy reliance on proven sires, which may subsequently lower the average relationship values for proven sires. An analogous way to capture this dynamic is through the expected future inbreeding (EFI), which is approximately half the average genetic relationship between a sire and a random sample of active cows. The objective of this study was to assess the current average genetic relationship trend between active foreign and domestic sires and currently active Canadian Holstein cows using both pedigree and genomic information.

Materials and Methods

Data

Pedigree and genotype data were provided by Lactanet Canada (Guelph, ON, Canada) following the April 2024 evaluation release. In addition, herdbook records for active milk-recorded cows and heifers were provided. The reference population used for analysis was defined as active cows with a recorded test date in April 2024, as well as heifers up to 30 months of age that were registered in the herdbook and had no recorded left-herd date.

In total, there were 616,258 animals in the pedigree, which included all known ancestors for the genotyped animals traced back 20 generations. The pedigree completeness index (PCI) for all animals in the pedigree was estimated going back five generations and only animals with a PCI greater than or equal to 90% were retained for further analyses.

Genotype data were available for a total of 146,698 animals linked to the pedigree. Of these, 8,504 were bulls with birth year between 2000 to 2023 and 138,194 were active cows or heifers that had not been culled from the herd with birth year between 2010 to 2024. All animals had genotypes on the 50K SNP panel (Illumina Inc., San Diego, CA, USA). Quality control was performed to retain autosomal SNP with a call rate greater than 95%, a minor allele frequency (MAF) greater than 5%, and a difference between expected and observed heterozygosity less than 0.15 (Wiggans et al., 2009). In addition, only genotyped animals with a five generation PCI greater than or equal to 90% were included in the final dataset, resulting in 8,491 bulls and 131,139 active cows and heifers retained for further analyses.

Statistical analyses

Genetic relationship values between each animal and the reference group were estimated in accordance with the method developed by Van Doormaal et al. (2003). This was then modified to incorporate genomic information for estimation of GR-values. The first step was iterative estimation of progeny gene contribution of each animal to the reference population for the sire and dam separately.

$$C_{si} = \sum_{i=1}^{m} 1/2C_i\,;\ C_{di} = \sum_{i=1}^{m} 1/2C_i \qquad (1)$$

where C_{si} and C_{di} are the i^{th} individual progeny gene contribution to their sire and dam, respectively, C_i is the contribution of the i^{th} individual with an initial starting value of 1 for all active animals and 0 for all other animals, and m is the number of animals in the pedigree.

$$R_i = \begin{cases} C_i & if\ S_i = 0\ and\ D_i = 0 \\ 0.75C_i + 0.5C_{si} & if\ S_i \neq 0\ and\ D_i = 0 \\ 0.75C_i + 0.5C_{di} & if\ S_i = 0\ and\ D_i \neq 0 \\ 0.75C_i + 0.5C_{si} + 0.5C_{di} & if\ S_i \neq 0\ and\ D_i = 0 \end{cases} \qquad (2)$$

where R_i is the total value of the i^{th} individual that reflect the genetic relationship to the reference group, and S_i and D_i are the sire and dam of the i^{th} individual, respectively, and when 0 it indicates unknown parent. The R-value is then defined as:

$$Rval_i = \frac{R_i}{n} \qquad (3)$$

where $Rval_i$ is the percentage genetic relationship value of the i^{th} individual and n is the total number of animals in the reference group.

For the genomic relationship values, equation (1) was modified to use the realized marker-by-marker similarity between progeny and parent, instead of assuming the expected value of 0.5 used for pedigree. In this case, actual SNP genotypes were used to estimate the proportion of identical-by-state (IBS) alleles shared between parent and offspring.

Results & Discussion

The distribution of genotyped active cows and heifers is presented in Table 1. Notably, 45.7% of the reference population represents heifers that have not calved. Including heifers in the reference group is essential to represent the expected future breeding population of active females. Furthermore, approximately 7% of cows were seven years of age or older, suggesting that older animals tend to leave or are culled from the herd. This dynamic turnover contributes to ongoing changes in the genetic relationship values estimated within the population.

Among genotyped bulls, those under the age of four accounted for 30.7% of the total genotyped bulls (Figure 1). This attests to the impact of genomic selection, which allows for early selection of bulls rather than the expected five years for progeny proven time (Schaeffer, 2006). This could contribute to diversification of the pool of available bulls and influence the estimated genetic relationship values within the population.

Table 1: Age distribution of the reference group as of April 2024.

Age (year)	Number of active cows and heifers	Percentage of total
<1	3,394	2.6%
1	28,073	21.4%
2	28,499	21.7%
3	23,775	18.1%
4	18,264	13.9%
5	12,620	9.6%
6	7,947	6.1%
7	4,155	3.2%
8	2,169	1.7%
9	1,214	0.9%
10	527	0.4%
>10	502	0.4%

The average annual genetic relationship per year (Figure 2) trend showed a steady increase over time, mirroring the pattern observed in the estimated increase in pedigree inbreeding reported by Van Doormaal (2024). Although, genomic relationship values were approximately 1.5 times higher than the pedigree relationship values, both increased in parallel from 1990 to 2024. Specifically, R-values increased from 14.6% in 2010 to 21.4% in 2024, while GR-values rose from 20.7% to 31.3% over the same period (Table 2).

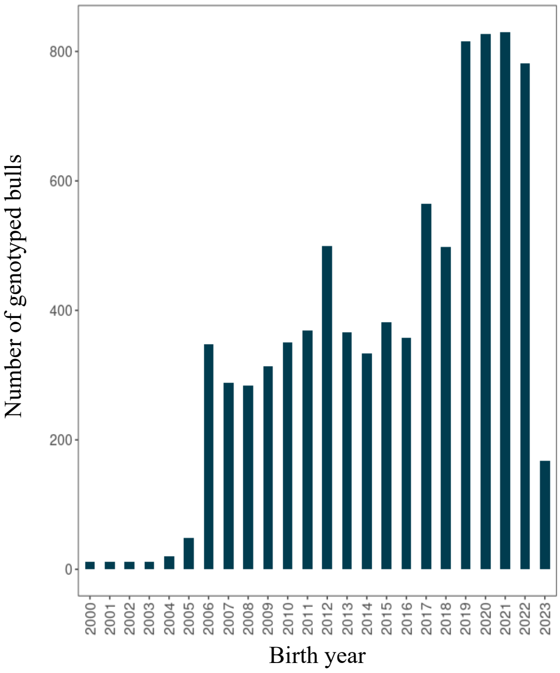

Figure 1. Distribution of the number of genotyped sires across birth year from 2000 to 2023.

Increase in both genomic and pedigree relationships suggest a concurrent increase in inbreeding levels within the population over time. Based on the estimated genetic relationships, the expected pedigree future inbreeding (EFI) and genomic future inbreeding (GFI) was estimated as half the R-values and GR-values, respectively. The EFI increased from 7.0% in 2010 to 10.6% in 2024, while GFI increased from 9.9% to 15.5%. These estimates aligned with the estimated pedigree inbreeding coefficients for the corresponding period and ranged from 6.6% to 11.1%.

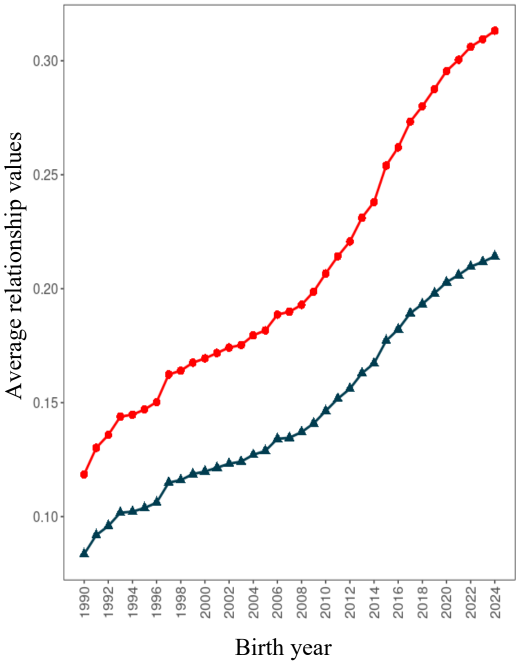

Figure 2. Annual average genetic relationship values per year based on pedigree and genomic information from 1990 to 2024. Blue line with triangles and red line with circles are pedigree and genomic relationship values, respectively.

Additionally, the observed correlations for pedigree inbreeding coefficients (Fped) with R-values and GR-values were moderately high at 0.77 and 0.76, respectively (Table 3). These findings suggest that genetic relationship measures can serve as a sufficient proxy of inbreeding levels and can be effectively used to inform mating strategies and selection decisions with the consideration of reducing inbreeding within the population.

Table 2: Average pedigree and genomic relationship values, pedigree and genomic future inbreeding estimated from relationship values and inbreeding coefficients across birth year from 2010 to 2024.

Birth Year	R-value (%)	GR-value (%)	EFI (%)	GFI (%)	Fped (%)
2010	14.6	20.7	7.0	9.9	6.6
2011	15.2	21.4	7.3	10.3	6.8
2012	15.6	22.1	7.6	10.7	7.1
2013	16.3	23.1	7.8	11.0	7.4
2014	16.7	23.8	8.1	11.6	7.5
2015	17.7	25.4	8.4	11.9	7.8
2016	18.2	26.2	8.9	12.7	8.1
2017	18.9	27.3	9.1	13.1	8.6
2018	19.3	28.0	9.5	13.7	8.8
2019	19.8	28.8	9.7	14.0	9.3
2020	20.3	29.6	9.9	14.4	9.7
2021	20.6	30.0	10.1	14.8	10.1
2022	21.0	30.6	10.3	15.0	10.4
2023	21.2	30.9	10.5	15.3	10.8
2024	21.4	31.3	10.6	15.5	11.1

This analysis identified the degree of relationship between each countries' bulls and the active Canadian cows and heifers. Results showed that bulls registered to the United States, Belgium, and the Netherlands had the highest average genetic relationship with the Canadian reference population, with R-values of 20.8%, 20.7%, and 20.2% and corresponding GR-values of 30.4%, 30.1%, and 29.3%, respectively (Table 4).

Table 3: Correlation coefficients between pedigree and genomic relationship values and pedigree inbreeding.

	R-value	GR-value	Fped
R-value	1		
GR-value	0.99	1	
Fped	0.77	0.76	1

These findings indicate frequent exchange of genetic materials between these countries and Canada. In contrast, two decades ago, bulls from Spain (11.4%), Japan (9.4%), and Italy (9.4%) had the highest R-values with active Canadian cows in 2004, which may indicate a shift in bull selection (Van Doormaal et al., 2005). Moreover, in 2004, the average Canadian bull R-values with active Canadian cows was estimated to be 11.7% and currently in 2024 it has increased to 19.4%, indicating an increase in genetic relatedness and, by extension, inbreeding. Additionally, a wider range of R-values (4.2% to 11.4.%) was observed between international bulls and active Canadian cows in 2004, which had substantially narrowed by 2024 (17.1% to 20.8%). This pattern indicates that the Holstein breed across countries has become increasingly similar over time, which is likely due to the frequent international exchange of genomic material and the widespread adoption of genomic selection.

Table 4: Properties of countries with genotype bulls that are connected with the active Canadian cows and heifers.

Country	Number of Bulls	Number of Daughters	R-value (%)	GR-value (%)	EFI (%)	GFI (%)
CAN	3,022	50,107	19.4	28.1	9.7	14.0
AUS	14	902	19.1	27.3	9.6	13.7
BEL	10	8	20.7	30.1	10.3	15.1
CHE	50	361	20.0	29.1	10.0	14.6
CZE	10	0	17.1	24.3	8.5	12.2
DEU	121	461	19.6	28.4	9.8	14.2
DNK	21	57	17.5	25.3	8.8	12.6
ESP	5	50	19.4	28.0	9.7	14.0
FRA	82	108	19.1	27.7	9.6	13.9
GBR	49	415	19.4	27.9	9.7	13.9
HUN	19	3	17.4	24.9	8.7	12.4
ITA	111	571	19.0	27.5	9.5	13.7
NLD	225	3,944	20.2	29.3	10.1	14.7
USA	4,745	57,120	20.8	30.4	10.4	15.2

The average genetic relationship values varied across bulls, ranging from approximately 10% to 25% for R-values and 14% to 36% for GR-values (Figure 3). This variation highlights the opportunity for strategic selection of bulls that are less related to the reference population to manage and minimize inbreeding levels. Additionally, AI companies can support this effort by diversifying bull selection pools and offering sires that are less genetically related to specific herds or producers. Figure 4 presents the genetic relationship values of the top 100 lifetime performance index (LPI) bulls, with R-values ranging from 19% to 25% and GR-values from 28% to 36%, reflecting a considerable genetic contribution to the population. This is expected because bulls with high LPI tend to have more daughters and shared ancestry, which ultimately increases their genetic relationship with the active cow and heifer population.

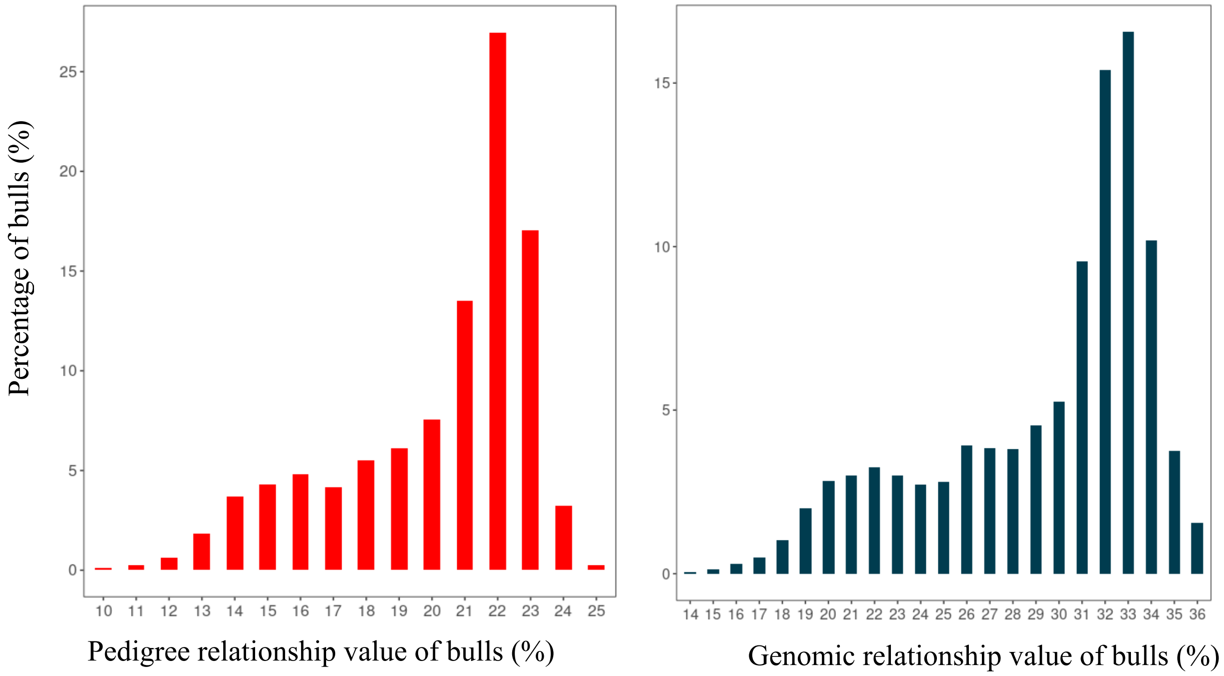

Figure 3. Distribution of relationship values of genotyped bulls with the active cows and heifers in the Canadian Holstein population using pedigree and genomic information.

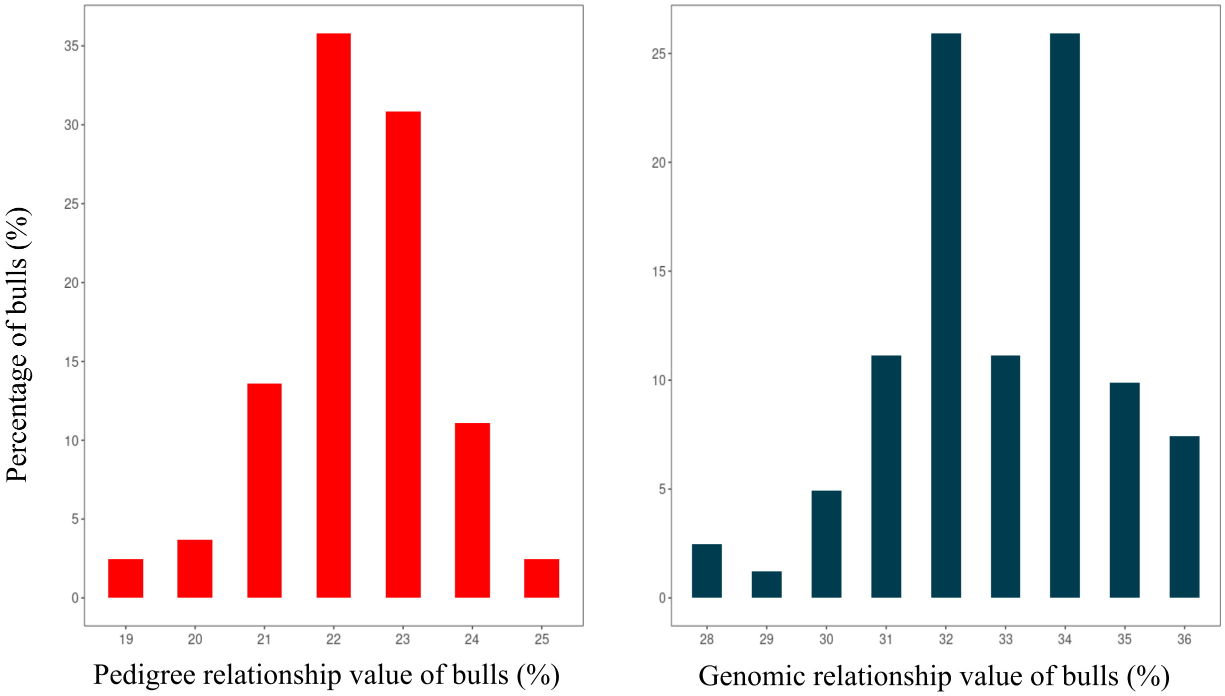

Figure 4. Distribution of relationship values for the top 100 LPI bulls in April 2024 with the active cows and heifers in the Canadian Holstein population using pedigree and genomic information.

Conclusions

Genetic relationships within Canadian Holsteins have steadily increased over time, corresponding with the observed rise in inbreeding levels. Additionally, genetic relationships between international bulls and active Canadian cows and heifers have increased, with differences between countries narrowed. The United States remains the major contributor of bulls to the active Canadian population. Among available bulls, variation exists in the genetic relationships with the active cows and heifers. This variation affords the opportunity to select less related bulls that could help manage and minimize inbreeding at the population level without trading-off the desired genetic gain. Finally, preventing the continued rise in inbreeding will require collaborative efforts from academia, AI companies, and producers.

Acknowledgments

We gratefully acknowledge all funding and support for the Resilient Dairy Genome Project (www.resilientdairy.ca/funders-and-partners/), as administered by Genome Canada, Genome Alberta, Ontario Genomics, Genome Quebec, and Genome British Columbia, as well as from the Net Zero Dairy Genome Project (//www.ndgp.ca/), as administered by Genome Canada, Ontario Genomics, Genome Alberta and Genome Quebec. Christine Baes also gratefully acknowledges support from the Natural Sciences and Engineering Research Council of Canada (NSERC, Ottawa, ON, Canada) Canada Research Chair and Discovery programs. As per the research agreement, researchers maintain independence in conducting their studies, own their data, and report the outcomes regardless of the results. The decision to publish the findings rests solely with the researchers.

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Improving the Stability of the Test-Day Repeatability Model for Production Traits in Italian Brown Swiss

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Abstract

The genetic evaluation of Brown Swiss cattle has undergone a significant methodological improvement with the introduction of a new algorithm designed to enhance the accuracy and stability of genetic indices for productive traits. This innovation addresses issues observed in previous models, particularly the significant variability in early evaluations of genomic bulls. The updated model incorporates a classification system for herds based on productivity differences between primiparous and multiparous cows, ensuring more precise genetic assessments. The new approach has resulted in greater stability in genetic indices and reduced the impact of initial data distribution biases. This advancement strengthens the reliability of genetic evaluations, supporting breeders in achieving their productivity goals.

Key words: Brown Swiss, Test days, Stability, primiparous, production traits, genomic

Introduction

Genetic evaluations in Italian Brown Swiss have been based on a test-day repeatability model since the early 2000s. This system was designed to support evaluations in a diverse environment with many small and mountain herds (Dal Zotto 2000). Genomic selection was introduced in 2011 (Rossoni 2009), and since 2019, the single-step genomic evaluation based on deregressed EBVs has been adopted (Vicario 2016).

However, over time, several issues related to index instability have emerged, particularly for widely used young genomic bulls. This instability undermines breeder confidence and complicates selection decisions.

This study investigates potential sources of instability and proposes a revised model incorporating a herd-level classification to better account for structural differences in herd productivity.

Materials and Methods

Data from routine national evaluations were analyzed to identify patterns and potential biases affecting the stability of early genetic indices.

We investigated several potential sources of instability, including:

– A high proportion of short lactations
– Predominantly first-parity records
– Cows calving at very young ages

However, none of these factors were found to be the actual source of the observed instability. In addition, we considered the non-random distribution of daughters across herds with varying management levels as a possible source of bias. As a first step, we tested whether heterogeneity of variance across herds could be responsible for the observed instability. However, this hypothesis was ruled out, as the observed pattern was exactly the opposite of what would be expected under variance heterogeneity. Typically, such heterogeneity leads to an overestimation of bulls whose daughters are mostly in high-producing herds. In contrast, in our case, bulls with daughters in high-producing herds appeared underestimated.

This led us to focus more closely on the average production difference between first- and later-parity cows within herds, which could interact with the distribution of daughters and contribute to the observed instability. To address these, a new herd-level classification was introduced based on the average milk yield gap between primiparous and multiparous cows in the previous three years period. Three levels were defined:

- High: Top 25% herds with the largest production differences
- Medium: Middle 50% of herds
- Low: Bottom 25% with smallest differences

This level was included in interaction with year, lactation number, age at calving, days in milk, and pregnancy status in the linear model:

$$y = htd + Ye \times L \times nlat \times age \times dim \times prg + pe + a + e$$

Where:

htd = herd test day
Ye = quinquennium of production
L = herd level based on production gap
nlat = number of lactations
age = age of calving
dim = days in milk
prg = days of pregnancy
pe =permanent environmental
a = additive effect
e = error.

As shown in Figure 1, the thresholds used to classify herds into Low, Medium, and High groups remained relatively stable until around 2010. After that point, particularly for the upper threshold separating Medium and High herds, a marked upward trend can be observed. This indicates an increasing divergence over time, with High-level herds showing a progressively larger milk yield gap between primiparous and multiparous cows compared to the others.

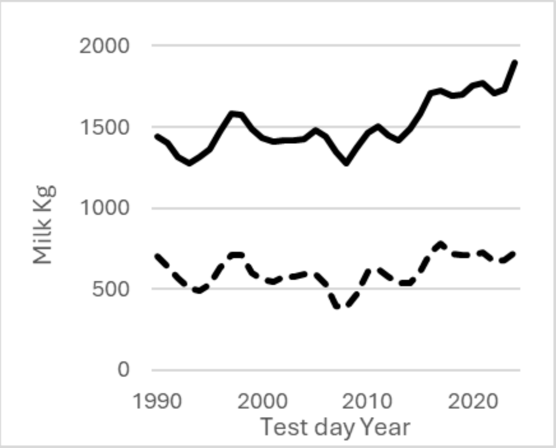

Figure 1. Herd average milk yield gap (kg) between primiparous and multiparous cows across herds classified Low (below the dashed line), Medium (between the dashed and solid lines) and High (above the solid line)

Results & Discussion

The updated model improved the stability of EBVs by reducing the influence of biased early data distributions. As shown in Figure 2, correlations between subsequent evaluations increased, and the advantage of the new model becomes more evident as the time interval between evaluations grows, reaching a difference of up to 0.03 when comparing evaluations four years apart.

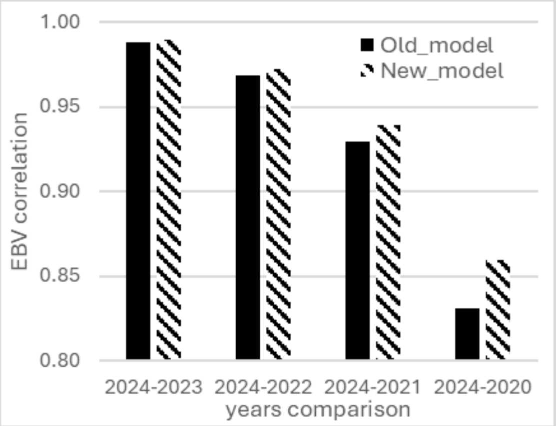

Figure 2. Correlations between estimated breeding values (EBVs) for sires across consecutive years, comparing the new model (white bars with diagonal hatching) and the previous model (solid black bars).

The updated model shows its greatest advantages in improving the stability of genetic evaluations for young bulls. As illustrated in Figure 4, which highlights the bulls with the largest changes in EBV between their first publication and the most recent one, the new model consistently produces smaller variations compared to the previous approach.

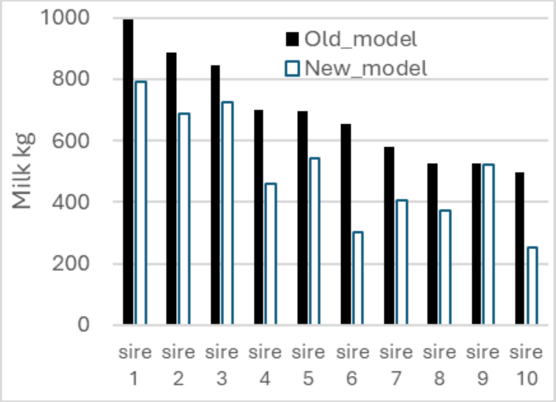

Figure 4. Difference between the first daughter-based evaluation and the most recent available evaluation for the 10 sires with the largest changes. Solid bars refer to the previous model, while white bars indicate the new model.

This increased stability is largely due to the model's improved handling of non-random herd distribution, a common issue when genomic bulls are initially used intensively in high-performing herds. By classifying herds according to the productivity gap between primiparous and multiparous cows, the new model incorporates both management level and its interaction with parity, thereby reducing bias and enhancing the reliability of early predictions.

Conclusions

Incorporating farm classification based on production differences between primiparous and multiparous cows into the genetic evaluation model substantially improves the reliability of early evaluations in the Italian Brown Swiss breed. This helps breeders make more confident decisions when selecting genomic bulls and enhances the credibility of the national genetic evaluation system.

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Foundational Review of U.S. Female Fertility Trait Evaluations

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Abstract

The Council on Dairy Cattle Breeding provides four female fertility evaluations for U.S. dairy producers: daughter pregnancy rate (**DPR**), cow conception rate (**CCR**), heifer conception rate (**HCR**), and early first calving (**EFC**). These evaluations were first introduced in 2004 for DPR, 2009 for CCR and HCR, and 2019 for EFC. Currently, these traits are expressed on six different breed bases: Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, and Milking Shorthorn. Over time, the data and methods used to calculate these traits have evolved in response to changes in availability, recording practices, and management systems. In recent tri-annual evaluations, unexpected season fluctuations have been observed in the Sire Estimated Breeding Values (**EBV**) of recently born bulls. The objective of this project was to identify the cause of these fluctuations and implement changes to improve stability across evaluations. In collaboration with the USDA Animal Genomics and Improvement Laboratory, this project also involves the research and development of a potential new trait to be added to the fertility evaluation, First Service to Conception (**FSC**), and re-estimation of genetic parameters for all five traits. Comprehensive tests were conducted to refine models, pre-adjustments, and data edits, including the use of both truncated and full datasets. Although data truncation showed promise in mitigating historical biases, it introduced higher variability in smaller breeds (Guernsey and Ayrshire). Additional tested changes included stricter calving year restrictions, improved data extraction procedures, updated CCR and HCR pre-adjustments, the inclusion of a days-in-milk covariate at first insemination for CCR and FSC, and the addition of a random herd-by-sire effect. Tests also examined whether modeling days open to pregnancy rate as a linear or non-linear trait, modeling traits as uncorrelated, performing unweighted analyses, or stricter convergence criteria of the traditional evaluation mixed model equations solver were appropriate. While the findings suggest that current methodologies provide a robust foundation, ongoing work is required to address the persistent slight negative trends reported in young bulls, where the underlying causes remain unclear. The project team is well-positioned to further enhance the stability of female fertility trait evaluations for U.S. dairy producers.

Key words: conception rate, pregnancy rate, predicted transmitting ability, breeding value fluctuations, trait stability

Introduction

Female fertility traits play an important role in dairy cattle breeding by offering insight into the reproductive performance of animals across diverse management systems. In the United States (**U.S.**), Daughter Pregnancy Rate (**DPR**) was introduced in 2004 (VanRaden et al., 2004; Van Raden et al., 2002), Cow Conception Rate (**CCR**) and Heifer Conception Rate (**HCR**) in 2009, and Early First Calving (**EFC**) in 2019, providing producers with tools to select for female reproductive performance (CDCB, 2025a;

Miles et al., 2023). DPR is calculated by a non-linear transformation of days open to pregnancy rate. The trait predicts the percentage of non-pregnant cows that will become pregnant during each 21-day period (VanRaden et al., 2004). CCR and HCR predict the ability to conceive at each insemination for lactating cows and maiden heifers, respectively. EFC predicts the animal's ability to alter their female offsprings age at first calving in days. All traits are scaled to their breed base of six breeds: Ayrshire (**AY**), Brown Swiss (**BS**), Guernsey (**GU**), Holstein (**HO**), Jersey (**JE**), and Milking Shorthorn (**MS**). Traits are often re-evaluated for continued improvement as management changes or more data becomes available (Hutchison et al., 2013; Miles et al., 2023; Wiggans et al., 2005).

In recent years, subtle but consistent seasonal patterns have been observed in fertility evaluations, especially in spring (April) tri-annual evaluations. The dairy industry raised concern after noticing that the estimated breeding values (**EBV**) of individual young bulls, particularly for DPR, were gradually, but consistently declining from evaluation to evaluation as these bulls accumulated more information. This is unexpected because some bulls should change upwards and some downwards. These trends prompted a deeper look into whether the current evaluation system reflected modern management practices and phenotypic data accurately, or whether aspects of the modeling might be contributing to these shifts.

To investigate, the Council on Dairy Cattle Breeding (**CDCB**), in collaboration with the United States Department of Agriculture Animal Genomics and Improvement Laboratory (**USDA AGIL**), launched a focused review in early 2024. The objective was not to overhaul the fertility evaluation system, but to understand the source of these trends, test updates to improve consistency, and determine whether any adjustments were needed.

This paper outlines the investigative process, highlighting data handling improvements, model refinements, and ongoing questions that emerged over the course of this project.

Materials and Methods

Data:

Phenotypic records are routinely extracted from the National Cooperators Database managed by the CDCB every tri-annual evaluation (OFFICIAL; CDCB, 2025b). In order to make a direct comparison against four OFFICIAL that have already been conducted, the test-runs utilize the database from December 2023 (**2312**), April 2024 (**2404**), August 2024 (**2408**) and December 2024 (**2412**) to extract new phenotype files. As of the most recent extraction, 2412, phenotypes were available for 94,528,060 DPR, 39,599,925 CCR, 13,311,667 HCR, and 37,300,141 EFC records. Heifer records, HCR and EFC, only have one record per animal whereas DPR and CCR can have up to 5 records per animal, one per lactation. Lactational CCR and HCR are aggregated values from events, usually inseminations or diagnostics, that happened within the lactation. The earliest available calving dates were January 1960 for DPR and EFC, December 2002 for CCR and October 2003 for HCR. Insemination dates required for CCR and HCR calculations were not collected nationally until 2003 (VanRaden et al., 2004).

Modeling:

For each test, traditional evaluations were generated by the fertility pipeline which includes data extraction, phenotype creation, pre-adjustments, and mixed model analysis. Animal effects were calculated using a pedigree-based BLUP with a multiple-trait, animal model. DPR, CCR, and HCR were developed using single-trait models, but were developed into a multi-trait model in 2015 (VanRaden et al., 2014). DPR, CCR, and HCR are treated as correlated traits (Kuhn et al., 2006; VanRaden et al., 2014), while EFC is treated as uncorrelated. These tests followed the same steps as the traditional evaluation conducted during the OFFICIAL.

Test Scenarios:

Two sets of scenarios were developed for testing. The first included changes applied to the full datasets (CHG), and the second used the same changes, but truncated the historical DPR and EFC records to December 2002 (CHG_TR). This allowed the same period of data to be used across all four traits. Both test scenarios were compared to traditional results from OFFICIAL.

Changes Applied to Tests:

Several changes were applied in both CHG and CHG_TR. A stricter calving date restriction was implemented so that only records with at least 365 days between calving and data extraction were included compared to the current edit of ≥70 days described by Hutchison et al., 2013. If calving dates or days open information were missing, those records were removed from DPR rather than estimated. Extraction programs were revised for efficiency and formatting. Pre-adjustments applied to individual inseminations were updated for both CCR and HCR and were estimated within each evaluation instead of fixed across evaluations. A DPR record was removed if the cow's sire was unknown, aligning it with existing edits for CCR, HCR, and EFC. A new covariable, days-in-milk at first insemination, was added to the CCR model. Additionally, the convergence criteria for the mixed model equations solver (described in VanRaden et al., 2014) were also made stricter.

A proposed additional trait, days from First Service to Conception (**FSC**), developed by USDA AGIL, was included in the tests evaluation pipeline and modeled alongside the other four traits. Updated variance components were estimated for all traits by USDA AGIL and University of Connecticut collaborators as part of this work. However, results related to FSC and the re-estimated variance components are not presented here and will be reported separately.

Additional changes were explored but were excluded from further testing due to limited benefits or failure to converge. These included modeling DPR as a linear function of days open, using unweighted analyses, treating all five traits as correlated, and including a random herd-by-sire effect.

Results

Impact of Data Edits:

Applying a stricter calving date restriction removed 2–4% of records from recent years across traits. Removing records with missing days open or calving dates for DPR had a minimal effect on overall record count but was important for ensuring consistency in how phenotypes were calculated. Removal of records with unknown sires reduced record counts primarily in earlier years and among smaller breeds, with little effect in Holstein data.

Pre-adjustment updates for CCR and HCR led to moderate shifts in phenotype distributions, especially in the most recent years, where older adjustment factors may no longer have reflected regional and seasonal differences in management. The inclusion of days-in-milk at first insemination as a covariate also influenced the distribution of CCR values, likely among high-producing herds where voluntary waiting periods may be longer. Research on voluntary waiting periods by herd and years is in-progress.

Phenotypic Trends:

Phenotypic trends by year of calving were broadly consistent across OFF, CHG, and CHG_TR. For most traits, the use of truncated data slightly reduced phenotypic variability in early years but had limited impact in recent years. Among smaller breeds (Guernsey and

Ayrshire), the truncation of pre-2002 data for DPR and EFC led to a more noticeable reduction in available records and corresponding shifts in average values.

The updated edits resulted in smoother trends in recent years, especially for DPR and raw CCR (cow conception rate without pre-adjustments applied). Raw CCR or raw HCR values were easier to interpret and more transparent in terms of seasonal or year-based shifts. However, these trends without pre-adjustments on individual inseminations also showed greater variability, especially in recent years when data volume is lower. The application of updated pre-adjustments within each evaluation test helped reduce this instability and produced smoother trends over time.

EBV Trends:

The EBV of young cows with phenotypes were averaged by birth year and segmented by their sire's breed. Figure 1 has 9 graphs of OFFICIAL (top), CHG (middle), CHG_TR (bottom) and 2312 vs. 2404 (left), 2404 vs. 2408 (center), and 2408 vs. 2412 (right) for Holstein DPR by year of birth.

Across all test scenarios, the applied changes did not substantially alter the consistency of these breed-level averages. The seasonal fluctuations originally observed in these figures, especially in April evaluations, remained present to some extent but were not worsened by the new edits or data truncation.

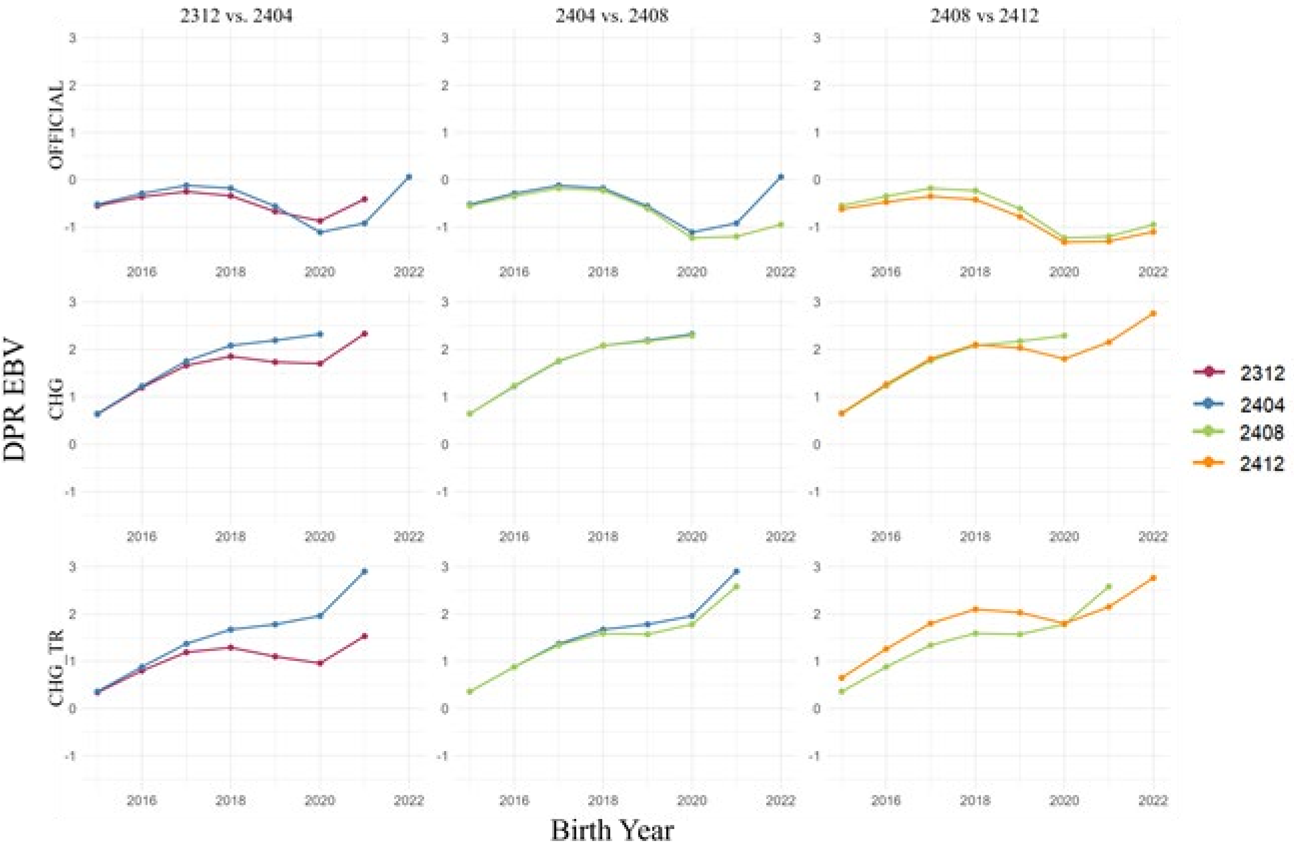

Figure 1. Mean daughter pregnancy rate (DPR) estimated breeding value (EBV) of young cows with Holstein sire official tri-annual evaluations (OFFICIAL; top), full data set with changes applied (CHG; middle), and truncated dataset with changes applied (CHG_TR; bottom) for multiple evaluations: December 2023 (2312), April 2024 (2404), August 2024 (2404) and December 2024 (2412).

Sire EBV Trends:

Although not shown in this report, future work will focus more directly on trends in the EBV of male animals. The industry concern prompting this investigation was centered on recent declines in DPR among young bulls. The exploratory analyses presented here did not fully resolve that concern but laid the groundwork for evaluating where those trends originate whether from the data, model assumptions, or something else.

Discussion

The goal of this review was to understand whether changes to the data pipeline or model structure could explain the seasonal variation observed in EBV for female fertility traits. While the test scenarios introduced several improvements, the comparisons among OFF, CHG, and CHG_TR suggest that the core evaluation system is already relatively robust, and that no single edit tested fully accounts for the observed patterns.

Across most traits and breeds, the CHG scenario which applied updated edits and model refinements without removing historical data showed the greatest internal consistency. Phenotypic trends were smoother, and changes to the pre-adjustments and model covariates helped reduce irregularities in CCR and DPR that often appear in more recent years. The edits removed relatively few records overall but targeted potentially less reliable data such as data including missing calving dates or undefined sires.

The CHG_TR scenario, in contrast, introduced greater variability, particularly in the smaller breeds. Truncating DPR and EFC records prior to 2002 ensured a uniform time range across traits, but the loss of early data reduced the sample size enough to destabilize trends for breeds like Guernsey and Ayrshire. For Holstein and Jersey, the impact of truncation was smaller, though not negligible. These results suggest that while historical data may introduce bias, it also contributes information for estimating trends, especially in populations with less data.

None of the edits tested in CHG nor CHG_TR substantially changed the EBV trends which reflect mean EBV of daughters grouped by their sire's breed. While these figures have been useful for monitoring population-level trends, they are not a substitute for direct evaluation of individual young male animals. The continued presence of seasonal fluctuations in these plots, even after updates, indicates that the source of variation may lie elsewhere.

The investigation also highlighted a recurring challenge in fertility evaluations: edits and model refinements often improve internal consistency but do not necessarily resolve the deeper patterns observed in young animal EBV. The inclusion of more refined covariates, like days-in-milk at first insemination, potentially helped account for some management-driven variability in CCR, but did not have an effect large enough to shift overall trends. Similarly, pre-adjustments estimated within each evaluation for CCR and HCR produced more stable results, but did not fully explain the seasonal fluctuations of interest.

The updates applied in CHG improved the evaluation pipeline and represent meaningful refinements. However, they did not resolve the underlying concern of declining EBV with consecutive evaluations in recently born bulls. Truncation (CHG_TR) introduced more variability than it removed and may be better suited for targeted applications rather than as a universal solution.

Conclusions

This project reviewed and tested a range of updates to the U.S. female fertility evaluation pipeline, with the goal of improving stability and addressing concerns about seasonal trends in the EBV of recently born bulls. While these trends remain an interest, the changes tested here did not appear to be the direct cause.

Edits implemented in the CHG scenario including stricter calving date filters, updated pre-adjustments, and improved handling of incomplete records contributed to smoother trends in phenotypes and improved consistency in recent years. These changes strengthened the overall foundation of the system and are candidates for future implementation. However, the comparison with CHG_TR showed that truncating historical data can introduce additional variability, especially for smaller breeds. This suggests that while older data may have some unanticipated effect, it continues to play a stabilizing role in multi-trait fertility evaluations.

Although the updates improved internal consistency and addressed specific improvement opportunities in the evaluation process, they did not resolve the seasonality of the trend observed in young bull EBV. Further work is needed to explore this issue more directly, particularly by evaluating how the actual EBV of male animals change across evaluations and whether changes in herd management, data recording practices, or model assumptions are contributing to the trend.

The results presented here represent a step forward in refining female fertility trait evaluations, but additional investigation is needed to fully understand and resolve the ongoing patterns observed in young animals.

Acknowledgments

The authors would like to thank U.S. Dairy Producers, member sectors and collaborators, as well as the USDA Animal Genomics and Improvement Laboratory (AGIL), and the University of Connecticut for contributions to this project. The support and input from an internal CDCB team were essential throughout the review process.

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Female Inclusive MACE for Improved Genetic Evaluations in Small Populations: A Special Case for Ayrshire Dairy Herds

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Abstract

Small reference populations hinder prediction accuracy in minor breeds, thereby limiting genetic progress. In response, the World Ayrshire Federation (WAF) initiated an international collaboration through the Interbull Centre (IBC) to enhance genomic evaluations for Ayrshire-based populations across countries. This initiative is primarily to ensure breed sustainability by addressing the challenge of limited reference population sizes in individual countries, which affects the reliability of national genomic evaluations. The aim of this study is to adapt IBC's Multiple Across-Country Evaluation (MACE) to include female estimated breeding values (EBVs) alongside male data while leveraging the Interbull Data Exchange Area (IDEA) and GenoEx-GDE platforms for data management.

The participating countries initially include Australia, Canada, Colombia, the United Kingdom, New Zealand, South Africa, and the United States. A total of 22,383 genotyped Ayrshire animals (4,403 males and 18,880 females) have been identified, with Canada contributing 46% of the genotypes. Adding cow data is envisioned to accelerate reference population growth, boost genotyping returns and improve the reliability of both national and international genetic and genomic evaluations over time. The first phase of implementation involves integrating cow EBVs into MACE while sharing genotypes among participating countries using the GenoEx-GDE platform. The second phase envisions extending these evaluations through InterGenomics for interested countries and those that cannot perform their own national genomic evaluations. This collaboration paves the way to faster growth of reference population sizes and improves genetic and genomic prediction accuracy for not only Ayrshire sustainability, but also the opportunity exists for other small breed populations like Guernsey, among others.

Key words: Ayrshire, genomic evaluation, MACE, small populations, international evaluation

Introduction

As genotyping costs continue to drop, the number of genotyped animals worldwide is on the rise. This creates an opportunity to significantly accelerate genetic gain through genomic evaluations on both national and international levels. However, the reliability of genomic predictions depends heavily on the size of the reference population, which links genotypic and phenotypic information. For numerically small dairy breeds such as Ayrshire, Guernsey, and others, a limited national reference population remains a persistent challenge that restricts the potential gains from genomic selection (Mäntysaari et al., 2010; Wiggans et al., 2011).

Traditionally, international genetic evaluations for dairy breeds have relied on the MACE system implemented by the IBC, which integrates bull evaluations from national genetic evaluation centres (Schaeffer, 1994). However, MACE has typically excluded cow EBVs, omitting valuable data that could enhance prediction accuracy. With IBC's data sharing platforms, such as the Interbull Data Exchange Area (IDEA) and GenoEx-GDE, it is feasible to exchange raw genotype data and expand genomic reference sets across countries under agreed-upon terms.

In this context, the WAF, in partnership with the IBC, initiated a collaborative project to investigate the possibility of including cow data into MACE. We hypothesise that incorporating female EBVs into the MACE system while leveraging the IDEA and GenoEx-GDE infrastructures will enable broader, more reliable international evaluations. This work holds potential not only for Ayrshire sustainability but also as a model for other numerically small or geographically dispersed dairy breeds. This paper outlines the proposed approaches and data management strategies for an international Ayrshire-based genetic evaluation that includes female EBVs, building on existing IBC services such as MACE and thus the future possibility of InterGenomics.

Materials and Methods

The first step of the project involves the modification of the existing MACE pipeline to incorporate female EBV records. This involves evaluating the accuracy of current de-regression methods for female data and ensuring no information overlaps between bulls and cows. Contributing organisations will supply female EBVs and pedigree data, alongside bull EBVs, while the Interbull Centre will conduct the necessary research to adapt, test, and validate the MACE pipeline and its outcomes.

Data Collection and Management

Data for the proposed research will include pedigree, genotypes, and EBVs (for both cows and bulls), managed through the IDEA and GenoEx-GDE platforms. Pedigree data utilises the Interbull International ID format, ensuring consistency across countries. Genotypes will be stored in GenoEx-GDE, with organisations controlling data-sharing permissions. Cow EBV and pedigree data uploaded to IDEA will undergo quality checks and verification of data integrity, together with bull data, before being included.

In 2023, participating countries shared genotype counts with CDCB, revealing a total of 22,383 genotypes for Ayrshire animals, with Canada contributing 46% of the total (Table 1). These data form the basis for evaluating reference population sizes and potential genomic evaluation improvements when females are added.

Table 1: Number of Ayrshire genotypes by sex and participating country

Country	Female	Male	Total
Canada	8,670	1,806	10,476
United States	3,107	1,973	4,180
South Africa	2,761	19	2,780
New Zealand	2,105	41	2,146
United Kingdom	1,175	468	1,643
Australia	1,062	96	1,158
Columbia	N/A	N/A	N/A
Total	**18,880**	**4,403**	**22,383**

Source: Brian Van Doormaal, 2024

MACE with Female EBVs

MACE, a multi-trait evaluation treating each country-trait combination as a separate trait, uses de-regressed proofs from national evaluations. Including female EBVs requires modifications to account for heterogeneous variance and potential double-counting of information. Proposed changes include adjusting data verification and checking programs in IDEA and establishing the threshold criteria for cow data inclusion (e.g., minimum number of daughters, herds, and status). Preliminary estimates of descriptive statistics from the data are expected to follow. Additionally, the research phase of the project will involve the investigation of the technical aspects below:

- The best method to deregress cow EBVs for MACE
- Adjustments to the MACE model
- Possible bias, double counting
- Changes in reliability
- Accurate conversion equations that include females

InterGenomics

After a successful MACE evaluation that includes cow data, international EBVs will be provided to the participating countries (Figure 1). For those countries that cannot perform national genomic evaluations, and those interested in international genomic evaluations, they might then have an opportunity to participate in a new InterGenomics service from IBC. With InterGenomics, MACE EBVs are used as phenotypes, and together with GenoEx-GDE genotypes, international genomic breeding values and their reliabilities can be estimated and provided to countries that request the service. It is also possible to obtain information on duplicate genotypes, SNP conflicts, and parentage conflicts.

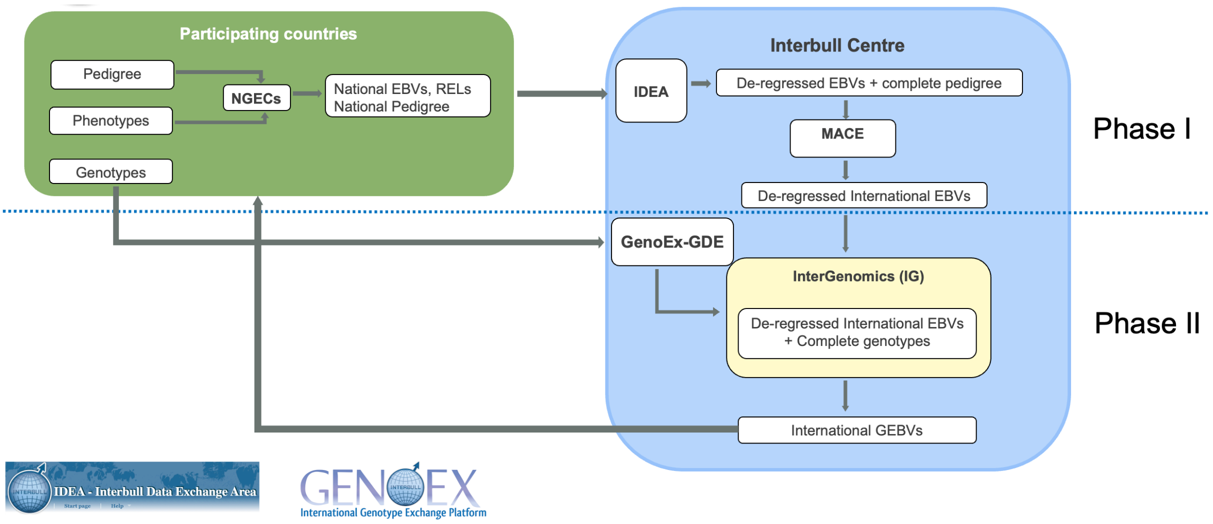

Figure 1. Proposed workflow showing the two implementation phases of including females in MACE project.

Results and Discussion

The international collaboration to enhance genomic evaluations for Ayrshire populations, as proposed by the participating countries involved in the World Ayrshire Federation (WAF), represents a significant step toward improving the reliability of genomic predictions for a breed with historically small reference populations in many countries. The initiative leverages the Interbull Centre’s infrastructure and expertise to integrate genotypic and phenotypic data from multiple countries, including Australia, Canada, Colombia, the United Kingdom, New Zealand, South Africa, and the United States. The preliminary data reveal a substantial pool of 22,383 Ayrshire genotypes currently available, with 84% being females, providing a robust foundation for expanding national genomic reference populations.

This section discusses the potential benefits, challenges, and considerations of the proposed methodologies, adapting MACE to include female EBVs while addressing their implications for Ayrshire breed sustainability.

Benefits of International Collaboration

The primary advantage of this collaboration is the significant increase in reference population size, which directly enhances the accuracy of genomic predictions. National evaluations for Ayrshire populations often suffer from limited reference populations, resulting in lower reliabilities for genomic estimated breeding values. By pooling genotypes from across countries, the reference population for bulls alone exceeds 2,900, a marked improvement over any single country's capacity. This aligns with findings from Bonifazi et al. (2020), who demonstrated that increasing the number of genotyped animals in international evaluations improves across-country genetic correlation estimates, thereby enhancing prediction accuracy.

For countries like Colombia and South Africa, where genomic evaluations are not yet established, this collaboration could enable the implementation of genomic selection, fostering breed sustainability. The inclusion of female EBVs in MACE further amplifies the reference population by incorporating cow data, which is particularly valuable given the high proportion of genotyped females, allowing faster genetic gains. Mäntysaari et al. (2011) showed that de-regressed cow EBVs can be effectively used in national genomic evaluations, suggesting potential for international applications.

The IDEA and GenoEx-GDE platforms facilitate secure permission-based data sharing, allowing countries to control access while benefiting from collective data (Figure 1). This infrastructure supports the standardisation of data formats and quality control, critical for ensuring evaluation consistency across diverse national systems (Nilforooshan and Jorjani, 2022).

Challenges in MACE with Female EBVs

Adapting MACE to include female EBVs presents technical challenges, particularly the risk of double-counting information from cows and their sires. This issue arises because cow EBVs may partially reflect sire contributions already included in MACE, potentially biasing results. Thus, careful de-regression methods are needed to mitigate bias. The Interbull Centre has outlined steps to address this, including defining thresholds for cow data inclusion, such as minimum daughter numbers, the type of cow, among others. These modifications involve research to develop de-regression techniques and validate data integrity. Additionally, heterogeneous variance across countries must be addressed, as differences in national evaluation models could affect the standardisation of EBVs (Nilforooshan and Jorjani, 2022).

Including cow data also necessitates updates to the IDEA database and verification programs, such as those in the *CheckProofsPara.py* and *verify_proofs.F90* programs to accommodate cow-specific metrics like genotyping status (codes "00" or "40" for non-genotyped or genotyped animals, respectively). These updates need to ensure that only valid female records are included and are subject to changes as the research goes on. While these changes are feasible, they require careful coordination with participating countries to establish consistent data submission protocols.

Strategic Considerations

Both methodologies require agreements on data sharing and result distribution, which involve political and legal considerations. The GenoEx-GDE platform allows countries to control data access, but consensus on sharing female genotypes and EBVs is critical. The IBC's experience with InterGenomics for Brown Swiss and small Holstein populations provides a model for establishing an Ayrshire-specific service, potentially managed by a Global Ayrshire Services Management Committee. This committee would facilitate ongoing discussions to address emerging opportunities and challenges, ensuring alignment with national priorities.

The choice to participate in MACE or InterGenomics with cow data depends on computational resources, data availability, and country preferences. MACE is less computationally intensive and leverages existing infrastructure, making it a faster option. However, InterGenomics may offer superior accuracy for small populations by directly incorporating genomic data (Bonifazi et al., 2023). A hybrid approach, combining MACE with InterGenomics, could balance feasibility and accuracy, as proposed for Brown Swiss (CDCB 2024).

Implications for Ayrshire Sustainability

This collaboration has the potential to transform Ayrshire breeding by enabling genomic evaluations in countries without national systems and enhancing existing ones. By maximising reference population sizes, the initiative addresses the breed's risk of declining relevance due to limited genomic progress.

Conclusions

This study presents a novel and collaborative approach to enhancing genomic evaluations for numerically small dairy breeds by adapting the IBC MACE system to include cows. Future research will focus on optimising the integration of female EBVs into MACE and on piloting InterGenomics services tailored to the Ayrshire population. Currently, a data call has gone out to participating countries, together with an agreement for collaboration that includes data sharing. This work may reshape how global evaluations are designed for underrepresented breeds.

Acknowledgments

For this project, we would like to acknowledge our partners so far, i.e. the World Federation of Ayrshire Breed Societies, CDCB, Lactanet Canada, DataGene, DairyNZ, AHDB, and SLU, to mention but a few and those that are soon to join us.

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Variance components estimation for methane emission in smallholders' dairy farms

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Abstract

Enteric methane (CH_4) emissions from cattle account for 70% of livestock GHG emissions in Sub-Saharan Africa. Also, climate change has impact on smallholder livestock-based food systems in terms of feed resources and emergence of new diseases. Direct selection for CH_4 is one of the approaches to mitigate the effects of climate change and this requires estimation of genetic parameters. Moreover, the amount of CH_4 emitted is influenced by the activity status (ACTs) of the cow such as feeding, ruminating, sleeping, and standing idle during time of measurement. The aim of this study was to evaluate CH_4 emissions under different activities, estimate variance components and compare accuracies of predicting CH_4 emissions using MIR information. The data consistent of over 14500 point-measurement of methane emissions measured by laser methane detectors with minimum duration of 3 minutes from 940 cows in 29 small-holders dairy farms in Ethiopia under various cow activities from July 2023 to March 2025. Records obtained under different ACTs for feeding, ruminating, sleeping, and standing idle were 2382, 7885, 660, and 3494 respectively. Pedigree information was also available for 435 cows with observation for CH_4 and the remaining 459 cows were genotyped using a 90k SNP chip. Overall average CH_4 production was 341 g/day. CH_4 production in feeding status was highest with 517 g/day on average. Pedigree BLUP (PBLUP), and single step combining both pedigree and genomic information (HBLUP) were applied to estimate variance components (VCs) using different modelling approaches. A repeatability animal model (full model (FM)) was fitted with ACTs, year-season, and average farm milk yield as fixed factors and permanent environmental effects a random effect in addition to animal. Also, records averaged within year-season subclasses (average model) were also analyzed with fixed effects of year-season and average farm milk yield and random effects of animal and permanent environmental effects. Heritability estimates for the FM were 0.09 (0.03), and 0.10(0.02) for PBLUP and HBLUP, respectively. The corresponding estimates for the average model were 0.14 (0.06), and 0.19 (0.04). For the indirect prediction of CH_4, a partial least square modelling approach was applied using milk mid-infrared data obtained in one-week period around the CH_4 measurements. The model with data restricted only to cows feeding gave higher prediction accuracy of 0.41 compared to 0.28 when using all data. In summary, heritabilities were low and consistent with published estimates, indirect predictions accuracy of CH_4 were moderate. In general, feeding status not only had the highest production average but also highest prediction accuracy and has influence on genetic parameters.

Key words: methane emission, animal activity, variance components

Introduction

Enteric methane (CH_4) emissions from cattle account for 70% of livestock GHG emissions in Sub-Saharan Africa years (GLEAM 2023), and it is of critical climate concern due to methane's short atmospheric lifespan of 12 years. Therefore, strategies to reduce enteric methane are vital for the 1.5°C global warming target and to mitigate the impact of climate change on the smallholder agri-food systems and livestock-based food systems in most developing countries in terms of feed resources, emergence of new diseases, increased levels of heat and humidity and related stresses. Studies have shown that methane emission is heritable and selective breeding for low emitting individuals through genetic selection is feasible (De Haas et al. 2021). Therefore, direct selection for methane is one of the approaches to mitigate the effects of climate change and this requires estimation of genetic parameters and variance components for methane and the capture of methane measurements. These recordings should be accurate and reflect overall methane production of individuals to maximize the accuracy of selection. The amount of the Methane (CH_4) emitted by cattle is not constant but varies with different activities because each activity changes the animal's rumen function, respiration rate, and gas release pathways (eructation, respiration).

Highest CH_4 production may occur during and after feeding. Rumen microbes ferment carbohydrates into volatile fatty acids and hydrogen which then methanogens convert hydrogen into methane. Methane peaks happen typically post-feeding especially after forage-rich diets (Rooke et al. 2014). Factors such as feed type (forage vs. concentrate), intake level, and feeding frequency strongly influence methane emission (Jiao et al. 2014).

Various technologies have been proposed to measure methane emission in cattle, each with different levels of accuracy, cost, practicality, and suitability for on-farm vs. research use (Sorg 2021). Most of these technologies record CH_4 when animals are in a particular state such as feeding or milking. These short time measurements of several minutes a day over a week are then generalized to estimate the methane production per day. Since animals may be different state of activities, such as feeding, drinking, milking, lying/resting, standing, walking or ruminating, CH_4 production may vary under different activities (ACTs). Therefore, to estimate an accurate amount of CH_4 production during a day, a comprehensive recording which includes these activities is needed for accurate predictions.

Methane recording in small holders' cattle farms is challenging and extra care needs to be taken for accurate and practical recording in scale. Laser Methane Detectors (LMD) are portable devices which has comparatively low purchase and running costs and results in only low-to-moderate behavioural changes of the animals but requires relatively high labour resources and has a moderate throughput in terms of the number of records per time (Sorg 2021).

Of the various technologies proposed to measure methane emissions in dairy cattle, the most commonly used include the GreenFeed and Fourier-transformed infrared (FTIR) breath analysers (sniffers) installed in feed bins (Sorg 2021).

Unlike GreenFeed or Sniffers which are installed in feed bins for recording methane only in the feeding status of cows, LMD can record methane during any cow activity, thereby providing the potential for a better estimation of overall methane produced by a cow.

As recording methane emission is still challenging and expensive, proxy traits such as milk mid-infrared (MIR) profiles are studied to indirectly predict CH_4 as an easy and cost-effective approach to record the trait. Training models for predicting methane emissions through proxy traits, relies highly on the accurate measurements of methane emissions under various the animal activities.

The aim of this study was to evaluate methane emissions under different activities, estimate variance components and compare accuracies of predicting methane emissions using MIR information under different ACTs.

Materials and Methods

About 14500 point-measurement of methane emissions from 940 cows recorded using handheld laser methane detectors in 29 small-holders dairy farms spanning a wide range of environmental conditions in Ethiopia were used for the study.

The duration of each point measurement was 3 to 5 minutes under various cow activities. Data was recorded at random times and days once or twice a month from July 2023 to March 2025. Each animal had between 2 to 32 records from farms with different management systems. The animals were of different ages, stages of lactation and were crossbreds resulting from crossing local cattle breeds with mostly Holstein and Jersey. After quality control 14421 records were analyzed and were recorded under different ACTs. A total of 2382, 7885, 660, and 3494 measurements were taken during feeding or ruminating or sleeping or standing idle respectively. Pedigree information was available for 435 cows with observation for CH_4 and 459 cows were genotyped using a 90k SNP chip.

Initially a fixed effect model consisting of ACTs, age at recording, breed proportion, lactation number, lactation stage, year-season, and average farm milk yield as management criteria were fitted to determine the factors with significant effect on methane.

Pedigree BLUP (PBLUP) and single step combining both pedigree and genomic information (HBLUP) were applied to estimate variance components (VCs) fitting significant effects from the fixed effect model.

An initial analysis indicated that repeatability of methane measurement was low at 0.26. Given this low repeatability, two sets of models were considered for estimation of genetic parameters. One set of models used the individual records of cows as the dependent variable or methane averaged year-season subclasses. The latter represents the average of subsequent measurements methane for a cow over a season of about 3-6 months and so mimics measurements of methane from other equipment such as the GreenFeed.

The full model (FM) including ACTs, year-season, and average farm milk yield as fixed factors and permanent environmental effects a random effect in addition to animal is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wp} + \mathbf{e}$$

where **y** is the observed CH_4 measurements, **b** is the vector of fixed effects, **a** is the random animal effect, **p** is the random permanent environmental effect, and **e** is the residual. Matrices **X**, **Z**, and **W** are the incidence matrices connecting fixed and random effects to the observations.

The model based on CH_4 records overaged a year-season subclasses (average model) consisted of fixed effects of year-season and average farm milk yield and random effects of animal and permanent environmental effect.

Indirect prediction of methane using MIR data

A corresponding 7714 milk mid infrared profiles from 608 individuals were available within ±7 days of LMD records. Out of 930 spectral points, three spectral regions were considered for the calibration process (968–1 577 cm-1, 1 720–1 809 cm-1, and 2 561–2 966 cm-1), resulting in the selection of 289 data points.

Savitzky-Golay filtering approach with 3rd order polynomial and a window size of 5 data points was used to improve the spectra resolution by eliminating constant baseline, and to obtain robust prediction models by restricting the insertion of bias into the model. We used Sgolay function implemented in R Signal package for this calibration process.

A partial least square modelling approach using 10 principal components to predict the methane emission using MIR information using R PLS package was used for prediction.

The full model to predict CH_4 by MIR information was as below:

CH_4 ~ MIR + milk fat% + milk protein% + body weight + milk yield.

The reduced model included only MIR information performed as below:

CH_4 ~ MIR.

A 5-fold cross validation approach was used so that one fifth of data was sampled randomly as validation set and the rest was used to train the model for prediction of methane emission by MIR data. One hundred sampling and prediction were performed and the average correlation value between predicted and actual measurements were calculated as accuracy of prediction.

Results & Discussion

Overall average methane production was 341 g/day. Methane production in feeding status was highest with 517 g/day on average. Average methane production under other activities were 296, 303, and 332 g/day for ruminating, sleeping, and standing idle, respectively.

Table 1: Summary statistics of data used in this study

trait	No. of animals	No. of records	mean	SD
CH4	940	14427	341	122
Milk yield	608	6423	12.5	4.7
Fat %	608	7714	2.97	1.44
Protein %	608	7714	3.36	0.6
MIR*	608	7714	-	-
genotypes	459	-	-	-

*Milk mid-infrared profiles.

The fixed effect model indicated that animal activity significantly influenced the methane production followed by age at recording.

Heritability estimates for the full model were 0.09 (0.03) for PBLUP and 0.10(0.02) for HBLUP models. Genotypic data increased the heritability estimates by only 0.01 which may be due to low genetic connectivity between animals in the pedigree. The corresponding estimates for the average model were 0.14 (0.06), and 0.19(0.04), which are higher than those from the full model, showing a significant difference in variance components in the two models with and without ACTs fitted. The heritability estimates are in the range of estimates from other publications for methane emission in cattle (Van Breukelen et al. 2023; Lassen and Løvendahl 2016; Ghavi Hossein-Zadeh 2022; Pszczoła et al. 2017). Moreover averaging over several point measurements as is common in other studies may increase the heritability estimates (Van Breukelen et al. 2023; 2022).

The partial least square modeling approach to predict methane emission by proxy traits using data restricted to only feeding activity had a higher accuracy of 0.41 compared to when using all data with accuracy of 0.28. studies show prediction of methane emission using MIR data in the range of 0.25 to 0.7 (McParland et al. 2024; Shadpour et al. 2022; Shetty et al. 2017). No study was found to compare prediction on methane emission recorded across various ACTs in cattle. Interestingly feeding status not only had the highest production average but also the highest prediction accuracy and a substantial influence on variance components estimation.

The accuracy of prediction using repeated records were studies to find the optimum number of records using LMD device. We examined animals with 1 to 12 records for the prediction. The results showed that 6 records per individual is the optimum number of records as show the highest accuracy while is value of accuracy is comparable to individuals having more records (Table 2).

Table 2: Changes in accuracy of predicting methane emission using milk mid infrared data in different number of records.

Average records	Accuracy	RMSE
1	0.24	168
2	0.28	149
3	0.29	133
4	0.37	124
5	0.39	122
6	0.45	116
7	0.47	116
8	0.45	109
9	0.45	107
10	0.45	106
11	0.46	105
12	0.45	106

*Residual mean square error.

Conclusions

The results indicate that heritability estimates for CH_4 using LMD were low at 0.09 to 0.14 but consistent with estimates reported using other more expensive equipment. The indirect prediction accuracies using MIR data were moderate and are encouraging. Furthermore, animal activities play an important role not only in terms of correctly measuring methane production but also influences estimation of genetic parameters and accuracy of prediction of CH_4 from MIR data.

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Quantifying the effective contribution of phenotypic records to genetic evaluations: a case study on enteric methane emissions

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Abstract

In the context of data sharing for genetic evaluation, such as enteric methane emissions in cattle, quantifying the effective contributions of phenotypic records to genetic evaluations is essential. This research introduces a framework for estimating the effective contribution of phenotypic records to genetic evaluations, using the concept of effective record contributions (ERCs). Our three-step approach involves: 1) computing reliabilities of a pedigree-based genetic evaluation using phenotypic information, 2) approximating ERCs due to own records, free of contributions due to relationships, from reliabilities of phenotyped animals using a reverse reliability algorithm, and 3) calculating the total effective contribution of phenotypic records as the sum of ERCs associated with all phenotyped animals. We apply this approach to a Dutch dataset comprising 187 219 records of weekly enteric methane emissions from 8 668 Holstein cows measured between March 2019 and April 2024. The pedigree spans five generations. Estimated heritability and repeatability were 0.18 and 0.47, respectively. We evaluate the effective contribution of weekly enteric methane emission records using: 1) the entire dataset, 2) a subset spanning until October 2023, instead of April 2024, 3) a dataset reduced by over 30% and limited to 20 records per animal, and 4) the entire dataset but considering the weekly enteric methane trait as an indicator trait genetically correlated to a hypothetical trait of interest with a heritability of 0.20 and a genetic correlation of 0.80. Results show that the entire dataset corresponds to 12 671 ERCs for the weekly enteric methane emission trait, which remains similar after reducing the number of weekly records by over 30%. The subset spanning until October 2023 corresponds to 10 870 ERCs. The reduction of ERCs can be explained by a smaller amount of records, but also by a smaller amount of recorded animals. Finally, when calculating the effective contribution to a correlated trait of interest, the entire dataset with weekly methane emission records corresponds to only 3 286 ERCs. Our approach provides a flexible framework for quantifying the effective contribution of phenotypic records to genetic evaluations. The proposed framework can be extended for optimizing data collection schemes when aiming to optimize the accuracy of genetic evaluations.

Key words: effective record contribution, phenotype, genetic evaluation, methane emissions

Introduction

Global climate changes pose significant threats to ecosystems and human societies, with rising temperatures and altered weather patterns. Livestock, particularly ruminants, contribute to greenhouse gas emissions, with enteric methane emissions accounting for a considerable portion of their emissions. Several strategies have been proposed to mitigate enteric methane emissions, including management practices, feed additives, vaccination, and genetic selection (Knapp et al., 2014). Genetic selection is particularly appealing due to its low implementation costs as well as its permanent and cumulative effects (Bishop and Woolliams, 2004). However, the success of genetic selection in breeding programs depends on several factors. A key factor is the definition of the breeding objective that will guide the designs of the breeding program and of the trait recording schemes (Banks, 2024). For traits difficult or expensive to measure, such as methane emissions in ruminants, a special attention for the design of the trait recording scheme is required, as the main limiting factor is the availability of sufficient phenotypes to create a reference population to estimate sufficiently reliable genetic parameters and breeding values required for informed breeding decisions.

Worldwide, research groups and breeding organizations are exploring the integration of methane mitigation into breeding programmes, requiring a clearly defined trait that is recordable, cost-effective, exhibits phenotypic variation, and is heritable. Recent studies (e.g., Manzanilla-Pech et al., 2021; van Breukelen et al., 2023) have explored various phenotyping methods and trait definitions. For instance, van Breukelen et al. (2023) compared daily and weekly means of methane production (grams/day) by GreenFeed units and of methane concentration (ppm) by sniffers, recorded on commercial dairy farms in the Netherlands. These differences in terms of phenotyping methods and trait definitions may limit the effectiveness of collaborations across research groups and breeding organizations, hindering the success of genetic selection for methane mitigation (Manzanilla-Pech et al., 2021).

In this context, the Global Methane Genetics (**GMG**) initiative (https://www.wur.nl/en/project/global-methane-genetics-initiative.htm) was launched in 2024 as a global initiative to accelerate genetic progress in reducing methane emissions in ruminants (Gredler-Grandl et al., 2024). The GMG initiative is coordinated by Wageningen University and Research-Animal Breeding and Genomics and funded by the Global Methane Hub and the Bezos Earth Fund. It aims to enhance genetic progress by establishing standard operating procedures for data collection, harmonizing protocols, facilitating the sharing of methane phenotypic and genotypic data, and increasing methane data recording in large and small ruminants across the world.

A key task of the GMG initiative is to first establish a GMG database for sharing data and protocols to support research, reference population expansion, breeding program design, and genetic evaluations for enteric methane reduction. The effective amount of information of phenotypic records provided by any party to the GMG database varies, impacting the composition of reference populations and the effectiveness of genetic evaluations and breeding programs with clearly defined breeding objectives. Factors that influence the effective amount of information of phenotypic records for a genetic evaluation are, of course, the number of phenotypic records provided, but also the definition of the recorded trait, the recording scheme and associated factors (e.g., the contemporary group sizes), and the accuracy of recording (reflected by heritability and repeatability), among others.

The objective of this research is to develop a framework to quantify the effective contribution to a genetic evaluation of phenotypic records submitted to a database. The framework considers the number of records provided, recording scheme properties, and the accuracy of recording (heritability and repeatability). Then, we apply our framework to a Dutch dataset including individual cow enteric methane emissions measured with sniffers in automatic milking systems (**AMS**). Results demonstrate the flexibility of our framework to quantify the effective contribution of phenotypic records to a genetic evaluation.

Materials and Methods

This section introduces the framework for quantifying the effective contribution of phenotypic records to genetic evaluations. We first define the concept of effective record contribution (**ERC**) to quantify the effective amount of information of phenotypic records. Second, we outline our proposed framework which leverages this concept. Finally, we present various scenarios assessing the framework using a Dutch dataset.

Effective amount of information

The concept of effective amount of information contributing to a genetic evaluation was introduced in the 80s to approximate reliabilities (**REL**) associated with estimated breeding values (e.g., Wilmink and Dommerholt, 1985; Robinson, 1986; Misztal and Wiggans, 1988; Meyer, 1989). The main goal of all these approaches is to summarize all information of an individual in a single value that is a diagonal element of a diagonal matrix $\mathbf{D}$ such that the diagonal elements of the inverse of ($\mathbf{D} + \mathbf{A}^{-1}\lambda$) are approximately equal to the prediction error variances obtained from the inverse of the absorbed coefficient matrix, ($\mathbf{Z'MZ} + \mathbf{A}^{-1}\lambda$), where $\mathbf{Z}$ is the incidence matrix relating phenotypes to animals, $\mathbf{M}$ is the absorption matrix including all fixed and random effects other than the random additive genetic effect, $\mathbf{A}^{-1}$ is the inverse of the pedigree relationship matrix, and λ is the ratio between the residual variance and the additive genetic variance.

Several measures have been proposed for summarizing the effective information, varying by application context. For sire evaluations, these measures focus on the records of progeny of a sire, and are called "daughter equivalent" (VanRaden and Wiggans, 1991), "effective number of daughters" (Wilmink and Dommerholt, 1985; Van Vleck, 1987), or even "effective daughter contribution" (Fikse and Banos, 2001). Similarly, for animal models, the proposed measures focus on the records of the animals themselves and are called "record equivalent" (VanRaden and Wiggans, 1991), "effective number of records" (Misztal and Wiggans, 1988) or "effective record contribution" (Meyer, 1989; Přibyl et al., 2013).

In line with VanRanden and Wiggans (1991), and for our context of quantifying the effective contribution of phenotypic records to genetic evaluations, we define one ERC as the amount of information contributed by a standard animal to its genetic prediction. This standard animal is defined as having one record and an infinite number of contemporary group mates. The total ERC for a standard animal (**ERC_total**) can be computed for a trait of interest using its REL, as follows:

$$\text{ERC\_total} = (1-h^2)/h^2 * \text{REL}/(1-\text{REL}),$$

with h^2 being the heritability of the trait of interest.

Notably, ERC_total is the sum of two components: ERC due to own records (**ERC_own**) and ERC due to relationships (**ERC_rela**). ERC_own represents the amount of information contributed by the own records of an animal, excluding information from relatives. ERC_own is influenced by factors such as the number of records, the contemporary group size, and the repeatability, among others (Misztal and Wiggans, 1988; VanRaden and Wiggans, 1991). In contrast, ERC_rela represents the amount of information contributed by relatives (through the parents and progeny) to the genetic prediction of an animal.

From a phenotypic dataset, algorithms for approximating REL and ERC_total involve accumulating information from an animal 's performance records or those of its relatives, and adjusting for finite contemporary group sizes, and potentially accounting for other fixed and random effects, such as random permanent environment effects in case of repeated records (Misztal and Wiggans, 1988; VanRaden and Wiggans, 1991; Tier and Meyer, 2004).

From REL, algorithms for approximating ERC_own and ERC_rela involve reversing REL or ERC_total (Harris and Johnson, 2010; Vandenplas and Gengler, 2012; Ben Zaabza et al., 2022). Such algorithms are often used to calculate appropriate weights for integrating deregressed proofs in a genetic or genomic evaluation (Harris and Johnson, 2010; Vandenplas and Gengler, 2012) or approximating genomic reliabilities (Ben Zaabza et al., 2022; Bermann et al., 2022).

Quantification of the effective contribution of phenotypic records to genetic evaluations

The framework for quantifying the effective contribution of phenotypic records to genetic evaluations consists in a three-step approach: (1) approximation of REL using a pedigree-based evaluation and the model associated with the phenotypic records; (2) estimation of ERC_own using a reverse reliability algorithm; and (3) calculation of the total effective contribution of phenotypic records by summing the ERC_own of all phenotyped animals.

Step 1: Approximation of pedigree-based reliabilities

The first step consists of approximating pedigree-based REL for the trait of interest using the provided phenotypic records, the pedigree, and the associated model and variance components. If the phenotypic records are available for an indicator trait correlated with the trait of interest, pedigree-based REL for the trait of interest can be approximated using genetic correlations between the indicator trait and the trait of interest, and the heritability of the trait of interest. For this study, pedigree-based REL are approximated using the Tier and Meyer (2004) algorithm, as implemented in the software MiX99 (Lidauer et al., 2013) and MiXBLUP (Vandenplas et al., 2022).

Step 2: Estimation of ERC due to own records

The second step involves estimating the ERC_own for animals with phenotypes, using pedigree-based REL of the trait of interest approximated in the first step. We assume these REL are from a univariate animal model including only the additive genetic and residual effects, and with variance components reflecting the heritability of the trait of interest. The estimation of ERC_own is performed using a reverse reliability algorithm, which aims to estimate ERC_own for phenotyped animals, independent of ERC due to relationships (that is, contributions through the parents and progeny). For this study, ERC_own for phenotyped animals are estimated using the reverse reliability algorithm proposed by Ben Zaabza et al. (2022) and based on Tier and Meyer (2004).

Step 3: Calculation of the total effective contribution of phenotypic records

The final step involves calculating the total effective contribution of phenotypic records to a genetic evaluation for a trait of interest by summing the ERC_own associated with all phenotyped animals. This measure allows for a comprehensive understanding of the overall impact of the phenotypic records on genetic evaluations.

Data and software

Enteric methane emissions were recorded by sniffers (WD-WUR version 1.0, Carltech BV) installed in AMS on 62 commercial dairy farms located throughout the Netherlands, between March 2019 and April 2024. Pedigree and other cow information were provided by CRV (Arnhem, the Netherlands). For further details on the data recording scheme and data editing, see van Breukelen et al. (2024).

After editing, the dataset used in this study comprises 187 219 records of weekly averaged enteric methane emissions for 8 668 animals in 62 herds. The pedigree, extracted from the animals with phenotypes, spans five generations and includes a total of 31 471 animals.

The same model and variance components as estimated by van Breukelen et al. (2024) are used in this study. It is worth noting that the model includes as random effects, additive genetic, within-lactation permanent environment, across-lactation permanent environment and residual effects. The heritability is 0.18, and the repeatability is 0.47, respectively (van Breukelen et al., 2024). The software MiXBLUP (Vandenplas et al., 2022) was used for approximating pedigree-based REL and for reversing REL to estimate ERC_own.

Description of the scenarios

Using the Dutch dataset with weekly enteric methane emissions, the proposed framework is investigated using four scenarios. The first scenario simulates an initial submission of the entire Dutch dataset (i.e., 187 219 records for 8 668 animals) to a database.

The second scenario simulates a subsequent submission of a second dataset to a database. In this scenario, the entire Dutch dataset is submitted to a database in two subsets: 1) the first subset includes 125 169 records up to October 2023 for 8 034 animals, and 2) the second subset includes 62 050 records from October 2023 to April 2024, including 634 animals that are not present in the first subset.

The third scenario simulates a subsequent submission of a dataset with additional records for animals that already have some records in the database. In this scenario, the entire Dutch dataset is submitted to a database in two subsets: 1) the first subset includes 125 484 records for all the 8 668 animals, with at most 20 records per animal, and 2) the second subset includes the remaining 61 735 records. It is worth noting that the subsets have a similar number of records in the second and third scenarios.

For the first three scenarios, the weekly enteric methane emission trait is considered as the trait of interest. In the fourth scenario, the weekly enteric methane emission trait is considered as an indicator trait genetically correlated to a hypothetical trait of interest with a heritability of 0.20 and a genetic correlation of 0.80. In this scenario, the proposed framework is applied on the entire Dutch dataset to estimate its effective contribution to genetic evaluations for the hypothetical trait of interest.

Results & Discussion

Scenario 1 – Entire Dutch dataset

Applying the first and second steps of the framework on the entire Dutch dataset results in ERC_own ranging from 0 to 2.7 ERCs per animal with phenotypes, with an average of 1.5 ERCs (Table 1). First, ERC_own equal to 0 indicate that certain animals and their associated phenotypic records do not contribute to a genetic evaluation at all. This can be explained, for example, by animals being part of contemporary groups that are too small.

Second, these ERC_own illustrate that the number of phenotypic records, ranging from 1 to 80 records per animal, and averaging 21.6 ERCs (Table 1) does not linearly translate to an effective contribution of a dataset to genetic evaluations. This can be also observed in Figure 1 showing that, beyond a certain threshold (approximately 8 to 10 records), ERC_own no longer increases substantially with an increase number of records, indicating diminishing returns on investment in collecting more records.

Table 1. Number of records, and effective record contributions due to own records for the entire Dutch dataset.

	Min.	Average	Max.	Total
Number of records	1	21.6	80	187 219
ERC_own[1]	0.0	1.5	2.7	12 671.4
ERC_total[2]	1.0	3.2	6.3	25 724.0

[1] ERC_own = Effective record contributions due to own records.

[1] ERC_total = Effective record contributions due to own records and due to relationships.

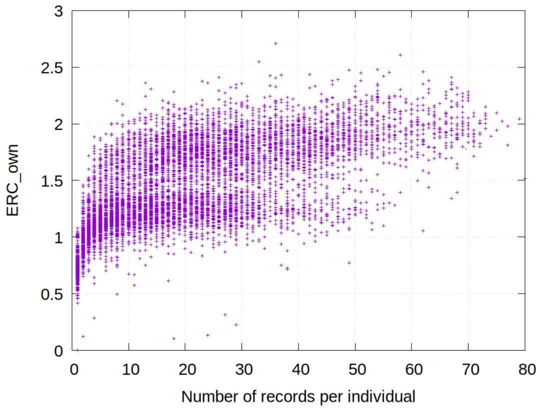

Figure 1. Effective record contributions due to own records (ERC_own) according to the number of records per animal.

Applying the third step of the framework on the entire Dutch dataset results in a total effective contribution of phenotypic records to a genetic evaluation for weekly methane emissions equal to 12 671 ERCs (Table 1). This sum of ERC_own is substantially lower than the number of records in the entire Dutch dataset (i.e., 187 219 records; Table 1), illustrating again that additional records do not linearly translate into effective contribution to genetic evaluation.

It is worth noting that ERC_own are lower than ERC_total because it include both contributions due to own records and due to relationships. Computed from the REL as ERC_total = $(1-h^2)/h^2$ * REL/(1-REL), the ERC_total range from 1 to 6.3 ERCs per animal with phenotypes, with an average of 3.2 ERCs (Table 1). The additional ERCs are due to relationships (ERC_rela), depend on contributions of relatives, and will therefore vary with a new submission of phenotypic records to the database.

Scenario 2 – Two subsequent submissions

For the second scenario, the entire Dutch dataset is divided into two subsets, with the first subset up to October 2023, and the second subset from October 2023 until April 2024. The first subset includes 8 034 animals and 125 169 records, and corresponds to a total effective contribution of phenotypic records of 10 870 ERCs, as estimated with the proposed framework.

The second subset dataset includes the remaining records of the entire Dutch dataset, as well as 634 animals that were not included in the first submission. Given that the entire Dutch dataset comprises 12 671 ERCs, we can estimate with the proposed framework that this second subset corresponds to 1 801 ERCs, calculated as the difference between the total effective contribution of the entire Dutch dataset (i.e., 12 671 ERCs) and the total effective contribution of the first subset (i.e., 10 870 ERCs). This effective contribution of the second subset can be explained by the additional records and also by the addition of 634 newly recorded animals.

Scenario 3 – Two submissions with no new animals

The framework is further investigated by dividing the entire Dutch dataset into two submissions, such that no new animals with phenotypes are added to the database. The first subset comprises 125 484 records for 8 668 animals with at most 20 records per animal and corresponds to a total effective contribution of 12 137 ERCs. Notably, this total effective contribution is comparable to that of the entire Dutch dataset, despite this first subset representing only 67% of the entire dataset.

Given that the entire dataset comprises 12 671 ERCs, the second subset corresponds to 534 ERCs only, calculated as the difference between the total effective contribution of the entire dataset (i.e., 12 671 ERCs) and the total effective contribution of the first subset (i.e., 12 137 ERCs). These total ERCs illustrate that this second subset will have a limited contribution to a genetic evaluation that already includes the first subset, even if it includes 61 735 records.

Scenario 4 – A hypothetical trait of interest

For the fourth scenario, the weekly methane emission trait is considered as an indicator trait genetically correlated to a hypothetical trait of interest with a heritability of 0.20 and a genetic correlation of 0.80. Applying the proposed framework on the entire Dutch dataset results in a total effective contribution of the Dutch phenotypic records to a genetic evaluation for this hypothetical trait of interest of 3 287 ERCs. This sum of ERC_own represents the effective contributions that flow from the indicator trait to the trait of interest through the genetic correlation.

A flexible framework

The different scenarios demonstrate the flexibility of the proposed framework to quantify the effective contribution of phenotypic records to genetic evaluations for a trait of interest. Our scenarios involve submission of datasets that include either records for a trait of interest, or records for an indicator trait.

Our framework can be easily extended to datasets that include records both for the trait of interest and for indicator traits. In such a scenario, pedigree-based REL for the trait of interest are approximated using phenotypic records of all traits with the first step of the proposed framework. Then, ERC_own are approximated for the trait of interest with the second step by reversing REL of the trait of interest assuming that they are approximated from an univariate model. The obtained ERC_own for the trait of interest include therefore ERC_own of the trait of interest, but also ERC_own of the indicator traits transferred through genetic correlations. Finally, the sum of all ERC_own of the trait of interest represents the effective contribution of all records of the trait of interest and of indicator traits to a hypothetical univariate genetic evaluation for the trait of interest.

Our framework can be also easily extended for estimating the effective contribution of phenotypic records to genetic evaluations of relatives of the phenotyped animals, such as selection candidates. By focusing on ERC_own of phenotyped animals, this study illustrates how individual phenotypic contributions can be aggregated to understand their cumulative impact on their genetic evaluations for a trait of interest. However, the aggregate effective contribution obtained from our framework does not reflect the effective contribution or phenotypic records to genetic evaluations of another group of animals, such as selection candidates. It has been shown that the accuracy of genomic prediction for selection candidates depends on the relationships among the reference animals and on the relationships between the reference animals and the selection candidates (Pszczola et al., 2012). Such contributions can be easily considered with our framework by estimating ERC_own for the selection candidates using the phenotypic records of the reference population with the second step of the framework, and then by aggregating these ERC_own to estimate the effective contribution of phenotypic records to genetic evasluations of selection candidates. The ERC_own of a non-phenotyped selection candidate is 0. However, the second step will estimate for selection candidates non-zero ERC_own that represent the effective contribution of relatives' phenotypic records to their genetic evaluations, without considering ERC_rela among them.

Finally, future research could aim to adapt the proposed framework for designing optimal recording schemes when aiming to optimize the accuracy of genetic evaluations. Such an optimization framework could integrate prior knowledge on the traits to be recorded (e.g., heritability, repeatability), but also prior knowledge on environment, management (e.g., seasons and herds), and recording costs (Banks, 2024). Future research could also aim to extend the proposed framework to consider genomic relationships, instead of pedigree relationships, in the first step of the framework. Considering genomic relationships will allow, for example, to estimate the effective contribution of phenotypic records of one breed to the genetic evaluation of another breed. That is currently not possible with the proposed framework because it is based on pedigree information only.

Conclusions

This study presents a flexible and comprehensive framework to quantify the effective contribution of phenotypic records to genetic evaluations using the concept of effective record contribution. This method relies on reversing reliabilities of estimated breeding values for a trait of interest by accounting for finite contemporary group sizes, and potentially other fixed and random effects, such as random environment permanent effects, as well as genetic correlations among the trait of interest and indicator.

Acknowledgments

This work is part of the Global Methane Genetics Initiative, funded by Bezos Earth Fund and the Global Methane Hub. The use of the HPC cluster has been made possible by the RegioDeal Foodvalley (through Shared Research Facilities Wageningen UR).

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Genomic prediction of methane emissions in Danish Holstein using single step and multi-trait prediction models

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Abstract

Enteric methane emissions from ruminants are a major contributor to global greenhouse gas emissions and pose a significant challenge to the sustainability of livestock production. To mitigate these emissions, breeding strategies have been mentioned as a promising tool, but prediction accuracies of methane emission traits are still limited by the size of datasets with records. Hence, using methane concentrations (MeC) in Danish Holstein cows as target trait, this study evaluated the predictive performance of pedigree-based BLUP (pBLUP) and single-step genomic BLUP (ssGBLUP) in univariate and multi-trait models, the latter including milk production traits. Previously, both ssGBLUP as well as multi-trait models have been shown to enhance prediction accuracies. The dataset included 1,744 primiparous (PP) and 2,989 multiparous (MP) cows from 15 Danish dairy farms, with over 600,000 daily records of MeC, fat yield (FY), and energy-corrected milk yield (ECM). Methane concentrations were measured using sniffers, and milk production data was acquired from milking robots and national milk recording data. At first, a pedigree-based variance component estimation revealed heritabilities between 0.17 (SE=0.03) for MeC in PP and MP cows to 0.38 (SE=0.06) for ECM in PP cows. Similarly, repeatabilities ranged from 0.32 (MeC, SE=0.01) to 0.81 (ECM, SE=0.01). Genetic correlations between MeC and production traits were positive but unfavorable, i.e., in a range from 0.15 (SE=0.13) between MeC and ECM in PP cows to 0.41 (SE=0.09) between MeC and ECM in MP cows, indicating a genetic antagonism between reducing emissions and maintaining milk yield. Prediction accuracies were generally higher for ssGBLUP compared to pBLUP models (up to 61.90% increase), and for MP cows compared to PP cows. Multi-trait models outperformed univariate models, particularly when phenotypic data for FY and ECM were available in both the reference and validation populations. The highest accuracy for MeC prediction in PP cows was 0.38 (ssGBLUP), while MP cows reached up to 0.51, both for the multi-trait model including both, ECM and FY. While incorporating FY and ECM improved MeC prediction, the unfavorable genetic correlations highlight the risk of compromising milk production when selecting for reduced emissions. Therefore, future breeding strategies should aim to expand methane phenotyping, develop methane traits independent of milk production, and implement multi-trait selection indices that balance environmental and economic goals. This study demonstrates the potential of multi-trait genomic prediction to enhance the genetic evaluation of methane emissions and supports its integration into sustainable dairy cattle breeding programs.

Key words: methane concentrations, single-step genomic prediction, multi-trait genomic prediction, predictor traits

Introduction

Methane is a potent greenhouse gas (GHG) with a global warming potential approximately 28 times greater than that of carbon dioxide (IPCC, 2024). At this, a significant proportion of anthropogenic methane emissions originates from enteric fermentation in ruminants, where microbial digestion of fiber in the rumen produces methane as a by-product (Knapp et al., 2014). Effective and sustainable mitigation strategies have thereby become imperative, given the fact that the European Union has committed to reducing GHG emissions by 55% by 2030 and achieving climate neutrality by 2050 (European Commission, 2019). Among the various approaches to reduce enteric methane emissions, such as feed additives and improved management practices, genetic selection offers a particularly promising long-term solution. This is, because unlike management-based strategies, genetic improvement can lead to cumulative and permanent reductions in methane emissions across generations (Knapp et al., 2014; Manzanilla-Pech et al., 2022a). However, the success of breeding programs targeting methane emissions depends on the availability of reliable phenotypic data for large populations of genotyped animals. Recent advances in phenotyping technologies have enabled the development of non-invasive, high-throughput methods for measuring methane emissions. Here, the sniffer method has gained popularity world-wide and measures methane concentrations (MeC) in the breath of cattle during routine milking or feeding (Garnsworthy et al., 2019; Lassen and Difford, 2020). This approach facilitates large-scale data collection at relatively low cost and has been shown to result in heritable phenotypes, with heritability estimates of MeC around 0.14 (e.g., Manzanilla-Pech et al., 2020). Despite these advances, accuracies of genomic prediction for methane emissions that are sufficiently high to enable genetic progress, remains limited, primarily due to the relatively small datasets. Different strategies to improve prediction accuracies of genomic evaluations, e.g., simultaneously exploiting genotypic, phenotypic and pedigree information, as in single step genomic prediction (Christensen and Lund, 2010), or by applying indirect information from correlated predictor traits, as in multi-trait prediction, have been proposed. Multi-trait genomic prediction methods are thereby exploiting genomic information from predictor traits that are highly correlated with the target trait and have earlier been shown to outperform univariate prediction methods (Calus and Veerkamp, 2011).

The objective of this study was to evaluate the predictive ability of pBLUP and ssGBLUP as well as univariate and multi-trait models to estimate genetic breeding values (GEBV) for MeC. Fat yield (FY) and energy corrected milk yield (ECM) were included as predictor traits in multi-trait models, since they were previously shown to be genetically correlated with methane emissions (Lassen and Difford, 2019). Moreover, these traits are directly recorded on the large scale, as they are part of the national milk recording scheme (Danish Cattle Database (SEGES, Skejby, Denmark)). To account for physiological differences between growing and mature animals, presumably leading to a different covariance structure between the applied traits, analyses were conducted separately for primiparous (PP) and multiparous (MP) cows.

Materials and Methods

Data collection

The dataset used in this study comprised daily records from 1,744 PP and 2,989 MP Danish Holstein cows, housed on 15 commercial dairy farms in Denmark. In total, 182,288 (PP) and 424,888 (MP) daily records were available for MeC, ECM and FY, collected between March 2021 and December 2024. Additional animal-level information, including pedigree, genotypic data, days in milk (DIM; 0–365 days), week in milk (WIM), parity, and age at first calving (AFC), was retrieved from the Danish Cattle Database (SEGES Innovation, Skejby, Denmark). The pedigree was pruned using the DMU trace software (Madsen, 2012) to include only animals with records and their ancestors born after 1970, resulting in a final pedigree of 47,383 animals. Genotypic data were provided by Nordic Cattle Genetic Evaluation (Skejby, Denmark). Most animals were genotyped using the Illumina BovineSNP50 BeadChip or imputed from lower-density panels. Imputation was performed by SEGES Innovation as part of routine evaluations resulting in a total of 46,342 single nucleotide polymorphisms available for the analysis. The majority, i.e. 97.31% of PP cows were genotyped, whereas the genotyping rate was lower for MP cows (73.00%).

Methane concentration measurements

Methane concentrations were recorded every second during the cows` visits to the automatic milking system (AMS) using sniffers, i.e., nondispersive infrared sensors (Guardian NG, Edinburgh Sensors, Livingston, UK) that were installed in the AMS feed bins and had a measurement range of 0–10 000 ppm for MeC. Since the sniffers themselves did not record animal identification numbers, which, however, are required to extract the abovementioned additional information about the cows from the Danish Cattle Database, a matching filter approach (Milkevych et al., 2022) was applied to link each measurement to the corresponding cow. Next, we applied a method to correct for background gas concentrations, head-lifting and diurnal variation, as described in detail in Løvendahl et al. (2024). For each visit, the mean MeC was calculated and then averaged across all visits per day to calculate daily MeC records, that are applied in this study.

Milk production traits

Daily milk yields (MY) were calculated from AMS data by using all milkings within the previous 96 hours, following ICAR standards (ICAR, 2023). Moreover, milk component data, i.e., fat percentage (FPCT) and protein percentage (PPCT), from monthly milk recordings were obtained from the Danish Cattle Database and linearly interpolated between two consecutive milk component recordings to generate daily values in alignment with the daily methane records. Next, daily FY and protein yield (PY) were computed by multiplying MY with FPCT and PPCT, respectively, in order to calculate ECM as $\mathrm{ECM\ (kg)} = 0.25 * \mathrm{MY\ (kg)} + 12.2 * \mathrm{FY\ (kg)} + 7.7 * \mathrm{PY\ (kg)}$, using the formula from Sjaunja et al. (1991).

Variance components and GEBV estimation

At first, variance components for MeC, ECM, and FY were estimated using the AI-REML algorithm implemented in the DMU software (Version 6, Release 5.4; Madsen and Jensen, 2014), thereby applying the following linear mixed model

$$y = X\beta + Za + Wpe + Ie.$$

Here, y is the vector of phenotypic observations for MeC, ECM, or FY. The vector β includes the overall mean and fixed effects, i.e., the WIM, as well as the AFC for PP cows (20–30 months), and parity (2nd to 8th parity) for MP cows. Moreover, a combined fixed effect of herd-year-season × AMS × sniffer box (HYS × AMS × sniffer) was included for MeC, while for ECM and FY, only HYS was modeled as a fixed effect. The corresponding incidence matrix that links the trait records to the fixed effect was denoted with X, and the terms a and pe are the random additive genetic as well as the permanent environmental effect with their corresponding matrices Z and W. The residual was denoted with e. It was assumed that these three terms follow a normal distribution with $a \sim N(0, A\sigma_a^2)$, $pe \sim N(0, I\sigma_{pe}^2)$, and $e \sim N(0, I\sigma_e^2)$, where A is the pedigree-based relationship matrix and I an identity matrix. Conversely, the additive genetic, permanent environmental and residual variance were denoted with σ_a^2, σ_{pe}^2, and σ_e^2. The heritability was calculated as $h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$, and the repeatability as $t = (\sigma_a^2 + \sigma_{pe}^2)/(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$. Genetic and phenotypic correlations were estimated from multi-trait analyses for MeC, ECM, and FY.

Table 1 Overview over the different scenarios performed per method (pBLUP, ssGBLUP).

Traits included in GEBV estimation	Type of analysis	Scenario name	Information included in	
			validation population	reference population
MeC	Univariate	1	-	MeC
MeC-FY	Bivariate	2a	FY	MeC, FY
		2b	-	
MeC-ECM	Bivariate	3a	ECM	MeC, ECM
		3b	-	
MeC-ECM-FY	Trivariate	4a	ECM, FY	MeC, ECM, FY
		4b	-	

GEBV: genomic EBV, pBLUP: pedigree-based BLUP, ssGBLUP: single-step genomic BLUP, MeC: methane concentrations, ECM: energy corrected milk, FY: fat yield

Next, different pBLUP and ssGBLUP methods, divided into seven univariate and multi-trait scenarios, were applied to estimate GEBV for MeC. An overview of the different scenarios can be taken from Table 1.

Briefly, the basic scenario, i.e., scenario 1, was a simple univariate scenario where phenotypes were only available for animals in the reference population. Multi-trait scenarios included FY, ECM, or both as predictor traits, each with two sub-scenarios: one where predictor trait phenotypes were available in both reference and validation populations, and one where they were restricted to the reference population. All scenarios were applied separately to PP and MP cows. GEBVs for MeC were estimated using DMU, applying the same fixed and random effects as in the variance component estimation. For ssGBLUP, the inverse of the H matrix was computed following Aguilar et al. (2010) and Christensen and Lund (2010):

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

where $\boldsymbol{G}$ is the genomic relationship matrix (VanRaden, 2008), computed using the invgmatrix software (Su and Madsen, 2011), $\boldsymbol{A}_{22}$ is the pedigree relationship matrix for genotyped animals, and $\omega = 0.8$ is the weight assigned to the genomic information.

Cross-validation groups

A 10-fold cross-validation strategy was used to assess the prediction accuracy of each scenario. Validation groups were constructed by sire using stratified random sampling to ensure balanced representation of paternal half-sibs. Sires were ranked by the number of genotyped daughters with MeC records, and one sire from each group of ten was randomly assigned to one of the ten folds. For each fold, MeC phenotypes were excluded from the validation group, and GEBVs were predicted using the remaining data as the reference population.

Accuracy calculation

Prediction accuracies were obtained following the approach of Manzanilla-Pech et al. (2020). At first, adjusted phenotypes for MeC were computed as the sum of the estimated genetic and permanent environmental effects from the full dataset, providing a single phenotype per animal. Then, accuracies for cross-validation group were calculated as the correlation between the adjusted phenotype and the GEBV for MeC divided by the following formula adapted from Mrode (2013) computed to calculate the accuracy for repeated records.

$$\text{Accuracy} = \frac{r}{\sqrt{\frac{nh^2}{\sqrt{1+(n-1)t}}}}$$

Here, the correlation between the adjusted phenotype and GEBV is denoted with r. The average amount of repeated records for each animal, specified per cross-validation group, is defined as n, and h^2 (t) is the heritability (repeatability) of MeC, taken from the variance component estimation (Table 2). Then, the accuracy for each scenario was calculated as the average of all cross-validation groups, and corresponding standard errors were obtained by dividing the standard deviation of accuracies across cross-validation groups by the square root of the number of validation groups, i.e., 10.

Results & Discussion

The estimation of variance components revealed moderate heritability estimates for MeC, FY, and ECM. Specifically, the heritability for MeC was estimated at 0.17 (SE=0.03) in both PP and MP cows. In contrast, ECM in PP cows exhibited the highest heritability at 0.38 (SE=0.06). These findings are consistent with previously reported estimates in the literature, such as heritabilities ranging from 0.26 to 0.37 for ECM (Li et al., 2018) and 0.14 for MeC (Manzanilla-Pech et al. 2020). Moreover, ECM in PP cows showed the highest repeatability with 0.81 (SE=0.01), while MeC was found to have low repeatability in both PP and MP cows, i.e., 0.32 (SE=0.01). Genetic correlations between MeC and production traits were moderate to weak and varied by parity. In MP cows, the genetic correlation between MeC and ECM was 0.41 (SE=0.09), and 0.37 (SE=0.09) between MeC and FY. In PP cows, these correlations were lower and accompanied by larger standard errors: 0.15 (SE=0.13) for MeC and ECM, and 0.18 (SE=0.13) for MeC and FY. Importantly, these positive genetic correlations are considered unfavorable, as they suggest that selection for increased milk production may inadvertently lead to higher methane emissions. A similar structure has been reported in previous studies, including a genetic correlation of 0.35 between MeC and ECM (Manzanilla-Pech et al., 2022b) and a correlation of 0.27 between GEBV for MeC and FY (Lopez-Paredes et al., 2020). A detailed summary of the estimated genetic parameters is provided in Table 2.

Table 2 Genetic parameters for methane concentrations (MeC), energy corrected milk (ECM) and fat yield (FY). Shown are the heritabilities (h^2), repeatabilities (t), and the genetic correlation (r_g) with MeC together with the corresponding standard errors in parentheses.

Trait	Primiparous			Multiparous		
	h^2	t	r_g with MeC	h^2	t	r_g with MeC
MeC	0.17 (0.03)	0.32 (0.01)		0.17 (0.02)	0.32 (0.01)	
ECM	0.38 (0.06)	0.81 (0.01)	0.15 (0.13)	0.24 (0.03)	0.74 (0.01)	0.41 (0.09)
FY	0.31 (0.06)	0.74 (0.01)	0.18 (0.13)	0.20 (0.03)	0.65 (0.01)	0.37 (0.09)

Regarding the different prediction scenarios, accuracies were generally higher for ssGBLUP than pBLUP models and for MP compared with PP cows. For PP cows, the increase from pBLUP to ssGBLUP was largest, i.e., 61.90% for the univariate scenario. Two scenarios resulted in a decrease in accuracies between pBLUP and ssGBLUP, i.e., -4.55% for scenario 3a in MP cows and -3.58% for scenario 3b in PP cows (Table 3). However, the observed difference was only small and might be owed to the generally rather small dataset. Moreover, we found an increase in accuracy from univariate to multi-trait models, but only when phenotypic information on predictor traits was available for the animals in the validation population. For PP cows, the highest accuracy of 0.38 was found for the ssGBLUP scenarios 4a (SE=0.03), 4b and 2b (SE=0.05, respectively), whereas the lowest accuracy was observed for the pBLUP scenario 1 (0.21, SE=0.04). In MP cows, prediction accuracies ranged from 0.31 (SE=0.04) in pBLUP scenario 2b to a maximum of 0.51 (SE=0.03) in ssGBLUP scenario 4a. A comprehensive overview of.

Table 3 Overview over the different pBLUP and ssGBLUP scenarios` accuracies (Acc), corresponding standard errors (SE, in parentheses), and difference between pBLUP and ssGBLUP (in %).

Traits included in GEBV estimation	Scenario	pBLUP		ssGBLUP			
		PP	MP	PP		MP	
		Acc (SE)	Acc (SE)	Acc (SE)	Difference to pBLUP (in%)	Acc (SE)	Difference to pBLUP (in%)
MeC	1	0.21 (0.04)	0.35 (0.02)	0.34 (0.03)	61.90	0.43 (0.03)	22.86
MeC-FY	2a	0.27 (0.03)	0.43 (0.04)	0.37 (0.03)	37.04	0.49 (0.04)	13.95
	2b	0.28 (0.05)	0.31 (0.04)	0.38 (0.05)	35.71	0.42 (0.03)	35.48
MeC-ECM	3a	0.24 (0.03)	0.44 (0.04)	0.36 (0.03)	50.00	0.42 (0.05)	-4.55
	3b	0.28 (0.05)	0.33 (0.04)	0.27 (0.04)	-3.58	0.41 (0.04)	24.24
MeC-ECM-FY	4a	0.28 (0.03)	0.44 (0.04)	0.38 (0.03)	35.71	0.51 (0.03)	15.91
	4b	0.28 (0.05)	0.33 (0.04)	0.38 (0.05)	35.71	0.43 (0.03)	13.16

GEBV: genomic EBV, pBLUP: pedigree-based BLUP, ssGBLUP: single-step genomic BLUP, MeC: methane concentrations, ECM: energy corrected milk, FY: fat yield, PP: primiparous, MP: multiparous

prediction accuracies across all scenarios is presented in Table 3

As anticipated based on previous results in dairy cattle (Hayes and Goddard, 2008; VanRaden et al., 2009), the accuracies of GEBV obtained using ssGBLUP were consistently higher than those obtained using pBLUP. This trend was observed across all scenarios and parities. Furthermore, multi-trait prediction scenarios yielded mostly higher GEBV accuracies compared to the univariate scenarios, which is in alignment with e.g. Tsuruta et al. (2011) for linear type traits. Notably, the improvement in prediction accuracy was most pronounced when phenotypic information for the predictor traits, ECM and FY, was available in both the reference and validation populations. This observation is consistent with the results of Pszczola et al. (2013), who reported enhanced prediction accuracy for dry matter intake when information on predictor traits was included in both populations. It is important to emphasize that the gain in GEBV accuracy for the goal trait in multi-trait genomic prediction depends on the extent of genetic correlations between the goal and predictor traits. Additionally, as noted by Jia and Jannink (2012), the relative heritability of the goal trait compared to the predictor traits also influences the extent of accuracy improvement. Specifically, the benefit of multi-trait prediction is more substantial when the goal trait has a lower heritability, as the contribution of genetically correlated traits becomes more impactful. Interestingly, both PP and MP cows exhibited increased prediction accuracies when FY and ECM were included in the genomic prediction models, despite the relatively low and imprecise genetic correlations between MeC and the predictor traits in PP cows. This may be explained by the larger difference in heritability between MeC and the predictor traits in PP cows, which could enhance the relative contribution of the predictor traits to the accuracy of MeC predictions.

Conclusions

In conclusion, using ECM and FY records can improve accuracy of MeC breeding values, especially for individuals without MeC records. However, it is important to keep in mind that the genetic correlations between MeC and both FY and ECM are unfavorable, indicating that selection for reduced methane emissions may reduce genetic progress in milk production. Since multi-trait prediction models are designed to exploit, but not to disentangle genetic correlations, selection based on these models may lead to genetic gains in MeC at the expense of economically important traits such as milk yield. Hence, further efforts are urgently needed to record methane emissions in more animals; to develop methane emission traits that are genetically independent from economically important, correlated traits like FY or ECM; and to design a multi-trait selection index including all economically important.

Acknowledgments

This study is part of GUDP-funded project, “ONIMIT: On-farm monitoring of methane from dairy cows is a prerequisite for the implementation of mitigation strategies on animal, farm and national level”. The authors declare that they have no competing interests.

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Sustainable dairy breeding: reducing methane emissions in the Netherlands and Flanders

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Abstract

Dairy cows contribute to the emission of methane (CH4), a strong greenhouse gas, into the atmosphere. Reducing CH4 emissions from dairy cows will lower the impact of livestock on global warming. Breeding could be an effective reduction method, and estimating breeding values was the objective of this work. The CH4 emission of 11,595 dairy cows in 89 Dutch herds was measured with sniffers in parts per million (ppm) of exhaled air. The CH4 emission of 397 dairy cows from 1 Dutch herd was measured in grams per day (g/d) of exhaled air using GreenFeed. CH4 measurements took place from 2019 to 2025 (sniffer) and from 2022 to 2025 (GreenFeed). All observations during a week on a cow were averaged into week observations. There were 226,449 week observations for ppm and 11,824 week observations for g/d. Genetic parameters were estimated with ASReml 4.2 using an animal multi-trait repeatability model. Heritabilities (h2) were 0.14, 0.14 and 0.19 for ppm and 0.34, 0.37 and 0.37 for g/d, for respectively parity 1, parity 2 and parity 3 and later (3+). Genetic correlations between different parities for ppm were 0.74, 0.47 and 0.79, and for g/d 0.73, 0.38 and 0.69, between respectively 1 and 2, 1 and 3+, and 2 and 3+. An overall breeding value was calculated for g/d based on traits in parity 1, 2 and 3+. By using a selection index, extra information was added to the overall breeding value in g/d. Traits in the selection index were kg milk production, kg fat production, feed intake and body weight with genetic correlations of respectively 0.39, 0.19, 0.20 and 0.09. The average CH4 emission of a dairy cow was 435 gram per day with a genetic standard deviation of 36 grams per day. The heritability of the trait, the size of the genetic standard deviation, and the fact that genetic correlations with health traits were estimated to be small, makes breeding an effective and powerful tool to mitigate CH4 emissions from dairy cattle in the Netherlands and Flanders. The overall breeding value for CH4 in grams per day is published in the Netherlands and Flanders from April 2025 onwards.

Key words: methane, methane emissions, sustainable dairy breeding, dairy, genetics

Introduction

In Europe, 10.8% of total greenhouse gas (GHG) emissions is from agriculture (EEA, 2023). Methane (CH4) is the second most important GHG, with a warming effect that is about 28 times more powerful than carbon dioxide. CH4 has a half-life in the atmosphere of 12.4 years, and reducing CH4 production leads directly to less GHG in the atmosphere and is therefore an effective measure against climate change (Cottle et al., 2011; Pachauri et al., 2014).

Around 85% of the CH4 coming from cows is formed by enteric fermentation in the rumen, and is emitted by breathing and belching. The other 15% of CH4 coming from cows is from manure storage and management.

Breeding is one of the CH4 mitigation strategies to reduce emissions from dairy cows.

Selecting animals that emit less CH4 than average as parents for the next generation, leads to a future generation that emits less CH4 than the previous generation. To know which animals have the lowest CH4 emissions, phenotyping animals on their CH4 emissions is necessary.

Phenotyping dairy cows on their CH4 emissions is challenging, since those techniques are often expensive and have low-throughput. Recently developed air analyzers, so-called 'sniffers', made it possible to phenotype dairy cows on their methane emission on a relatively large scale. Sniffers measure the CH4 emission of cows in parts CH4 per million of analyzed air. GreenFeeds are another CH4 measurement system, and are relatively high-throughput, although they can measure less animals compared to a sniffer, but with more accurate measurements. GreenFeeds measure the CH4 emission of cows in grams CH4 per day.

Materials and Methods

Data

A total of 89 herds, located all over the Netherlands, were selected to phenotype their cows with sniffers (Carltech B.V., Maarheeze, the Netherlands). The sniffers were located in the milking robot (automatic milking system, AMS), so cows were phenotyped during milking. Each herd had only one sniffer installed, so on herds with more than one AMS, there was only one AMS equipped with a sniffer. Because there were not enough sniffers to phenotype on all herds at the same time, sniffers rotated between herds. The first herds started phenotyping in 2019, and by 2025, all herds had a phenotyping period of at least two years.

The sniffer was not connected with the AMS, so observations from the sniffer were merged with AMS data afterwards to assign the sniffer measurements to the correct cow, based on date and time.

Next to the 89 herds that phenotyped their cows with sniffers, data was used from one herd in the Netherlands that phenotyped their cows with GreenFeeds (C-Lock Inc., Rapid City, South Dakota, USA). Three GreenFeeds were installed in this herd, and measurements started in 2022.

There is diurnal variation in CH4 emissions. Cows emit more CH4 after eating, and CH4 emissions are decreasing after a long period without eating. The diurnal variation is dependent on the management/ feeding strategy, and is therefore herd specific.

All sniffer observations were corrected for diurnal variation (van Breukelen et al, 2023). In addition to correction for diurnal variation, GreenFeed observations were also corrected for GreenFeed unit since there were three units on the farm where measurements took place. The correction for diurnal variation and unit was based on the estimated effects for θ and $unit_i$ according to formula 1:

$$y_{ijklmnop} = \mu + \sum_{j=1}^{1}(\sin j\theta 2\pi + \cos j\theta 2\pi) + unit_i + year*season_p + animal_k + dim_l + par_m + afc_n + e_o \quad (1)$$

where $y_{ijklmnop}$ is the measurement of CH4 emission, j is the order of regression, and in this analysis an order of 1 was used, θ is a decimal fraction of the time of measurement during the day following a 24-h diurnal cycle, $unit_i$ is the GreenFeed unit i within the herd, $year*season_p$ is year and season p, $animal_k$ is the kth animal, dim_l is days in lactation l, par_m is the mth parity, afc_n is age at first calving in months n and e_o is the residual error o.

The residual error was a random effect, the diurnal variation was a covariable using a fifth order polynomial, all other effects were fixed effects. The estimated effects for diurnal variation are shown in figure 1.

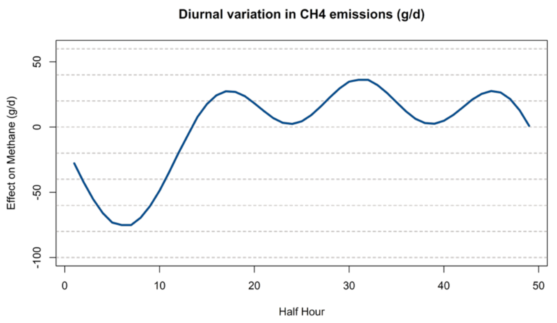

Figure 1. Diurnal variation in CH4 emissions (g/d) on herd with GreenFeed measurements relative to 0 a.m.

All measurements on a cow during a week were averaged into a week observation for both sniffer and GreenFeed.

In April 2025, 457,036 week observations for sniffer from 14,089 dairy cows on 89 different herds were available, and 11,889 week observations from the GreenFeed on 404 cows were available.

For the breeding value estimation, only herdbook animals were allowed and the minimum number of measurements in the week observation was four for sniffer and three for GreenFeed. After those selection criteria, 226,449 weekly sniffer observations from 11,595 cows with 1,380 different sires and 11,824 weekly GreenFeed observations from 397 cows with 154 different sires were selected for the breeding value estimation.

Parameters

Parameters were estimated for parity 1, parity 2 and parity 3+ (parity 3 and higher) and were based on 139,098 weekly sniffer observations and 9,974 weekly GreenFeed observations. The total number of cows was 7,175. The distribution over the parities was 38,934, 33,097 and 67,067 for sniffer observations, and was 5,450, 3,589 and 935 for GreenFeed observations, for respectively parity 1, parity 2 and parity 3+. All cows were at least 87,5% Holstein. Parameters were estimated using an animal model, including a H^{-1} pedigree-genomic augmented inverse matrix (Aguilar et al., 2010; Christensen & Lund, 2010).

Model

The statistical model used for methane emissions was split up into a model for parity 1 and parity 2 and a model for parity 3+. The model for parity 1 and parity 2 is given in formula 2:

$$Y1_{ijklmnopr} = HYS_i + DIM_j + AAC_k + HET_m + REC_n + INB_o + A_p + PME_l + Rest_{ijklmnopr} \qquad (2)$$

The model for parity 3+ is given in formula 3:

$$Y2_{ijklmnopr} = HYS_i + DIM_j + PAR_k + HET_m + REC_n + INB_o + A_p + PME_l + Rest_{ijklmnopr} \qquad (3)$$

In which:

Y1	observation on methane for cows in parity 1 and parity 2;
Y2	observation on methane for cows in parity 3+;
HYS	herd x year x season *i* (for sniffer observations) or farm x year x month *i* (for GreenFeed observations);
DIM	days in lactation *j*;
AAC	age at calving in months *k*;
PAR	parity number *k*;
HET	heterosis effect *m*;
REC	recombination effect *n*;
INB	inbreeding effect *o*;
A	additive genetic effect of animal *p*;
PME	permanent environmental effect *l*;
Rest	residual term *r* of that which is not explained by $Y1_{ijklmnop}$ and $Y2_{ijklmnop}$.

The effects A, PME and Rest were random, the effects HET, REC and INB were covariables, the other effects were fixed.

Correlations with other traits

Genetic correlations between the methane traits and traits in the Dutch/ Flemish total merit index were estimated using the MACE procedure. The MACE procedure can be used to estimate genetic correlations between deregressed sire estimated breeding values (EBVs) of different traits (Larroque and Ducrocq, 1999; Schaeffer, 1994). The initial function of the MACE procedure was to evaluate bulls for one trait across countries by Interbull (Interbull Centre, 2017).

The genetic correlations were estimated for both the overall breeding value for CH4 ppm and CH4 g/d. The breeding values for the methane traits were estimated using a single step approach, and only Holstein bulls with a reliability of at least 40% were used to estimate genetic correlations.

Results & Discussion

Model effects

Figures 2 to 4 show the effects of lactation stage, age at calving and parity number on methane emissions for gpd. These effects resulted from the model solutions.

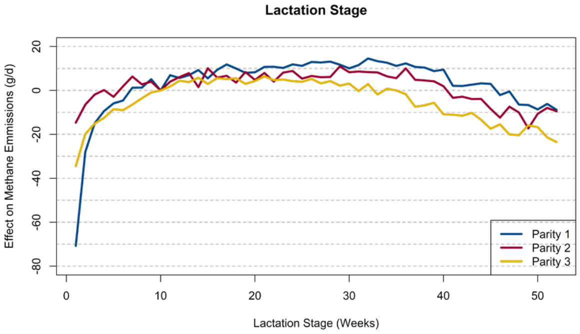

Figure 2. Effect of lactation stage in weeks on methane emissions in grams per day for parity 1, parity 2 and parity 3+. Solutions are standardized with week 10 within each lactation.

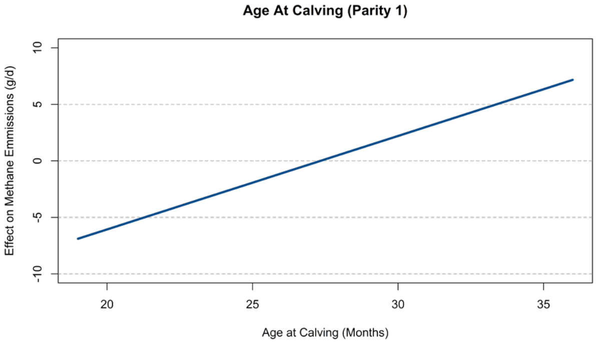

Figure 3. Trendline of solutions for effect of age at calving in months on methane emissions in grams per day for parity 1.

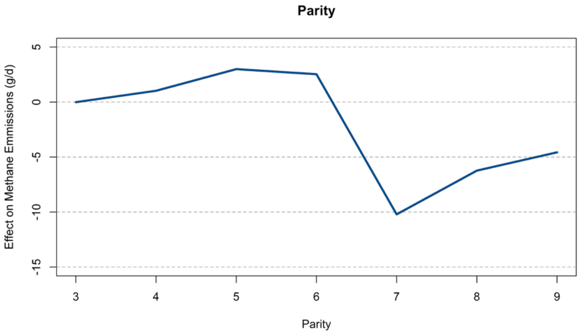

Figure 4. Effect of parity number on methane emissions in grams per day.

For lactation stage, the difference between the top of the graph in mid-lactation and the bottom of the graph in early and late lactation stage is about 30 grams per day. The estimates per week are relative to week 10.

Heifers calving at an older age have higher methane emissions. The effect of age at calving is about 15 grams per day difference between animals calving at a young and those calving at an old age. The effects are relative to a calving age of 27 months.

For parity number, animals in parity three, four, five and six have higher methane emissions compared to the older cows in later parities. The difference is about 10 grams per day. The effects in the figure are relative to parity 3.

Genetic parameters

Genetic parameters were estimated for weekly CH4 emissions in ppm measured with sniffers (CH4 ppm) and weekly CH4 emissions in grams per day measured with GreenFeeds (CH4 g/d) for parity 1, parity 2 and parity 3+. Table 1 shows the heritabilities, repeatabilities and genetic standard deviations of the methane traits. Table 2 presents the genetic correlation between parities for the methane traits.

Table 1. Heritabilities (h2), repeatabilities (r2), and genetic standard deviation (σ_a) for methane traits in parity 1 (par.1), parity 2 (par.2), and parity 3+ (par.3).

Trait	h^2	r^2	σ_a
CH4 ppm par.1	0.14	0.49	21.1
CH4 ppm par.2	0.14	0.55	23.3
CH4 ppm par.3	0.19	0.55	28.3
CH4 g/d par.1	0.34	0.60	43.2
CH4 g/d par.2	0.37	0.60	50.4
CH4 g/d par.3	0.37	0.60	50.4

The heritabilities in table 1 shows that CH4 emissions have a moderate heritability, with heritabilities between 0.14 and 0.19 for weekly measurements with sniffers and heritabilities between 0.34 and 0.37 for weekly measurements with GreenFeeds. Higher heritabilities for CH4 emissions measured with GreenFeeds are expected since measurements of GreenFeeds are of a higher quality.

The measurements for CH4 ppm are re-scaled and converted to a mean of 0, what makes that the standard deviations given in table 1 cannot directly be related to the measurements in ppm.

The genetic standard deviations of CH4 g/d in table 1 are based on the real observations. These are different from the genetic standard deviation of EBV CH4 g/d on an absolute scale, as that one is taken into account the reliability of the EBVs.

Table 2. Genetic correlations for methane traits between parity 1 and 2 (par.1-2), parity 2 and 3+ (par.2-3), and parity 1 and 3+ (par.1-3).

	genetic correlations		
trait	par.1-2	par.2-3	par.1-3
CH4 ppm	0.74	0.79	0.47
CH4 g/d	0.73	0.69	0.38

The genetic correlations in table 2 between parity 1 and parity 2 and between parity 2 and parity 3+ are considered as moderate to strong genetic correlations with values in the range 0.69 – 0.79.

Between parity 1 and parity 3+, the genetic correlations were lower and considered as moderate with a value of 0.47 for CH4 ppm and 0.38 for CH4 g/d. This lower correlation can be expected based on the estimated correlations between the other parities.

The genetic correlation between CH4 ppm and CH4 g/d was not estimated in this research, since the number of cows and observations was relatively small for CH4 g/d and there were no cows with observations on both CH4 ppm and CH4 g/d. This genetic correlation was already estimated for the Dutch population Holstein cows, based on a dataset with more CH4 g/d observations compared to this research, at 0.76 (van Breukelen et al., 2023). This genetic correlation was used in the genetic variance/covariance matrix between CH4 ppm and CH4 g/d for all combinations of parities.

Overall breeding values

Breeding values were estimated for both CH4 ppm and CH4 g/d for parity 1, parity 2, and parity 3+. An overall breeding value was calculated for both traits based on the EBV in parity 1, parity 2, and parity 3 with a weight of respectively 0.423, 0.288, and 0.227.

Correlation with other traits

The genetic correlations with the total merit index and the underlying traits of this index are presented in Table 3.

Table 3. Genetic correlations for overall methane traits with production, health, and conformation traits. (EBVs for methane traits: higher EBV is lower methane emission)

trait	CH4 ppm	CH4 g/d
NVI (total merit index)	-0.00	-0.06
milk production	-0.08	-0.39
fat production	-0.28	-0.19
protein production	-0.01	-0.18
longevity	0.12	0.03
udder conformation	-0.08	-0.01
feet & legs	0.02	0.04
fertility index	0.00	0.04
udder health index	0.05	-0.07
claw health	0.01	0.07

feed intake	-0.11	-0.20
body weight	-0.03	-0.09

Higher breeding values for methane reflect lower CH4 emissions, since lowering the CH4 emissions is desirable. Therefore, the genetic correlation with production is negative, meaning that a higher breeding value for production corresponds to a lower breeding value for methane. Animals that are more productive, will on average emit more CH4.

The correlations of CH4 g/d with the other traits are somewhat larger than the correlations of CH4 ppm with the other traits. This is expected given the fact that the GreenFeed is able to measure CH4 emissions more precisely compared to the sniffer.

All correlations are small, only the correlation between CH4 g/d and milk production can be considered as moderate. The largest genetic correlations for CH4 g/d are found with milk production (-0.39), fat production (-0.19), protein production (-0.18), feed intake (-0.20), and body weight (-0,09). The correlations with health and conformation traits are small and very close to zero, meaning that it is possible to breed for less CH4 emissions without affecting the health and conformation of the animals.

Selection index

Because the number of phenotyped animals is relatively small compared to other traits, the reliability of the EBVs is rather low. To increase this reliability, indicators traits are used in a selection index.

The methane trait used in the selection index is CH4 g/d because the unit of this trait makes it possible to calculate the genetic progress, reduction of CH4 emissions, as a quantity. This will also be the trait that is published to farmers and bull-owners. CH4 g/d does still contain information about CH4 ppm by using the genetic covariance between both traits.

Next to CH4 g/d, the other traits in the selection index are milk production, fat production, feed intake, and body weight. These traits have the largest correlations with methane, and that is also expected from a biological point of view.

Table 4 presents the genetic standard deviations and heritabilities of the traits in the selection index and the genetic correlations between them. The correlations between the traits are all estimated based on bull breeding values. The genetic standard deviations are based on the EBVs, with for relative EBVs a fixed genetic standard deviation of 4.5.

Table 4. Genetic standard deviations of EBV (σ_a) and heritabilities (diagonal) and *genetic correlations* (below diagonal) of the traits in the selection index.

trait	σ_a	CH4 g/d	milk production	fat production	feed intake	body weight
CH4 g/d (-)	4.5	0.56				
milk production (kg)	745	*-0.39*	0.58			
fat production (kg)	28	*-0.19*	*0.50*	0.57		
feed intake (kg)	1.37	*-0.20*	*0.56*	*0.67*	0.20	
body weight (-)	4.5	*-0.09*	*0.05*	*0.15*	*0.41*	0.60

The heritability for CH4 g/d, 0.56, is based on the heritability for the overall trait, which takes into account that there are multiple measurements on CH4 emissions, in multiple parities.

Daughter proven bulls will not profit much from the selection index. The reliability of their breeding value for CH4 g/d will, depending on the number of phenotyped daughters, increase with 0 to 3% by using the correlation structure with the other traits in the selection index.

Genotyped animals without progeny information on CH4 emission will increase their EBV with 5 to 10%, depending on their reliabilities for the traits in the selection index.

Publication

The breeding value that is published by CRV is overall CH4 g/d after the selection index. This is a relative breeding value with mean of 100, based on animals born in 2020, and a genetic standard deviation of 4.0 (assuming a reliability of 80%).

The mean CH4 emission for Dutch and Flemish Holstein cows is 435 grams per day (van Breukelen et al., 2023), with a genetic standard deviation of 36 grams per day. This genetic standard deviation is on the scale of relative breeding values and is standardized to 80% reliability, so 4 points breeding values is equal to 36 grams per day. The true genetic standard deviations are given in table 1.

Mating an average cow (EBV 100) with a bull with an EBV of 104 for CH4 g/d will, on average, result in offspring with EBV 102, which corresponds to 18 grams less CH4 emissions per day.

The average reliability for daughter proven bulls is 46.5%, with reliabilities up to 83%. Number of daughters is in the range of 1 – 576. The average reliability for genomic bulls is 32.2%, with reliabilities up to 43%. These reliabilities will increase as more animals are phenotyped in the coming years.

EBVs will be published for bulls with at least one phenotyped daughter, and for genotyped bulls if the bull-owners give CRV permission to do so. The EBV should have a reliability of at least 25%. If not, a parent average is calculated and published when the reliability of the overall EBV before selection index is at least 10%.

Cow EBVs are published for all cows with phenotypic information and all cows with genotypic information. If not, parent averages are calculated and published if the reliability of the EBV after selection index is at least 10%.

EBVs will only be estimated and published for Holstein Friesian (HF) cows, as there is only phenotypic data available from HF cows.

Conclusions

CH4 emissions in dairy cows can be measured large-scale. More than 14,000 dairy cows were phenotyped with sniffers in the period 2019-2025 on 89 Dutch herds, and more than 400 dairy cows were phenotyped with GreenFeeds in the period 2022-2025 on one Dutch herd.

CH4 emissions of dairy cows is a heritable trait. CH4 ppm has a heritability of respectively 0.14, 0.14 and 0.19, while CH4 g/d has a heritability of respectively 0.34, 0.37 and 0.37, for parity 1, parity 2, and parity 3+.

The EBV that is published is overall CH4 g/d. A mean EBV, 100, corresponds to a CH4 emission of 435 grams per day. The genetic standard deviation is 4 points and corresponds to 36 grams per day. Lower CH4 emissions are desirable, so EBVs above 100 reflects animals with below average daily CH4 emissions. Mating a cow with EBV 100 with a bull with EBV 104 results, on average, in offspring with EBV 102. The CH4 emission of the offspring will be, on average, 18 grams per day lower than her dam.

More productive animals tend to have a somewhat higher CH4 emission. So, animals with high EBVs for production tend to have lower EBVs for CH4 emission on average. However, genetic correlations with production are small to moderate (-0.39, -0.19, and -0.18 for respectively milk, fat, and protein), indicating that there is still enough variation between animals that makes it possible to select animals that are productive with lower CH4 emissions. Also higher feed intake and more body weight are related to somewhat higher CH4 emissions. So, animals with higher EBVs for feed intake and bodyweight tend to have lower EBVs for CH4 emission. Genetic correlations on EBVs are -0.20 and -0.09 for respectively feed intake and body weight.

Genetic correlations with health, longevity, and conformation traits are estimated to be small. So breeding on lowering CH4 emissions from dairy cows will not affect the health, longevity, and conformation of the animals.

The reliabilities for bulls are ranging from 25 to 83%, depending on their pedigree, daughter information, and genomic information. The average reliability is 46.5% for daughter proven bulls and 32.2% for genomic bulls. Reliabilities will increase when more cows are phenotyped.

Estimating breeding values for CH4 emissions made sustainable dairy breeding possible in the Netherlands and Flanders. Farmers can breed for dairy cows which emit less CH4 without compromising on production and health.

Acknowledgements

Setting up a breeding value estimation for CH4 emissions was part of the *Climate Smart Cattle Breeding* project. This project aimed at: 1. phenotyping dairy cows in the Netherlands on their CH4 emissions, 2. estimating breeding values for cows and bulls in the Netherlands, 3. developing tools to make the genetic level of CH4 emissions on a herd visible for farmers, and 4. encouraging farmers to reduce the CH4 emissions of their cows by selective breeding. The project was conducted from 2020 to 2025 and was funded by the *Dutch ministry of Agriculture, Fisheries, Food Security and Nature* (LVVN, The Hague, the Netherlands) and the project partners Wageningen University and Research (WUR, Wageningen, the Netherlands), FrieslandCampina (Royal Friesland Campina, Amersfoort, the Netherlands) and CRV (CRV u.a., Arnhem, the Netherlands).

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Advancing Genomic Evaluation for Methane Efficiency in Walloon Holstein Cattle towards Implementation

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Abstract

For several years, dairy cattle breeding in the Walloon Region of Belgium has increasingly focused on sustainability, including strategies for reducing methane emissions. Genetic selection provides a viable long-term approach to mitigating methane emissions while maintaining economic viability. The current study aimed to present a single-step genomic evaluation framework for methane efficiency (ME) based on predicted methane (PCH4) derived from milk mid-infrared (MIR) spectra and its integration into the existing genomic evaluation system for Holstein dairy cattle. The study incorporated data from 285 530 first-parity, 224 643 second-parity, and 160 226 third-parity Holstein cows across 1 520 herds. Genomic information from 9 631 animals, including 1 823 bulls, was integrated using a single-step GBLUP approach with a three-trait model (PCH4 across three parities). The predictive accuracy of the genomic evaluation framework was validated using a set of 2 038 youngest genotyped animals. Approximate genetic correlations (AGC) were calculated between PCH4 and 37 traits included in the Walloon breeding goal. Three methane efficiency (ME) indices were evaluated: relative ME based on production (RMEP), relative ME based on functionality (RMEF), and relative ME based on a global economic index (RMEG). The results demonstrated that the mean daily PCH4 ranged from 324 to 367 g/day, with mean daily heritability estimates between 0.20 and 0.23 for the first three lactations. The genomic prediction accuracy for PCH4-GEBV was 0.83. The AGC between PCH4 and the 37 traits ranged from -0.16 (milk yield) to 0.53 (fat percentage), highlighting the importance of balancing methane reduction with economic performance. Among the three ME indices, RMEG exhibited the most favorable balance, supporting its integration into genomic evaluations. Bulls with higher ME indices produced progeny with lower methane emissions, demonstrating the potential for genetic selection to contribute to sustainability goals. In light of these findings, we propose that INTERBULL considers methane for international genetic evaluations as many countries start to generate breeding values. These and other MACE breeding values would allow us to generate ME indices locally. Further discussions should focus on integrating reducing methane into breeding programs while maintaining productivity and functionality traits, as well as exploring strategies to incorporate direct methane measurements. Alternative thinking and use of tools like desired gain index will be required, but most important will be better knowledge about economic value of methane and its genetic relationship to other traits of interest. These initiatives will support sustainable dairy breeding strategies, aligning environmental and economic objectives for the future.

Key words: methane index, mid-infrared spectra, genetic correlation, single-step random regression

Introduction

Reducing methane emissions from dairy cattle is a critical component of sustainable livestock production. In the Walloon Region of Belgium, breeding programs have increasingly prioritized environmental sustainability alongside productivity. Genetic selection offers a long-term, cumulative solution to mitigate methane emissions without compromising economic performance.

Genomic evaluations for methane emissions faces three major challenges in a breeding context:

- Availability of adequate phenotypic data representing methane emissions.
- Development of an adapted genomic evaluation system.
- Reporting methane EBV to breeders in a way that allows to mitigate methane emissions while maintaining breeding for increased economic performances.

Therefore, the objective of this document was to report the latest on how the Walloon Region is overcoming these challenges in the development of a genomic evaluation system for methane efficiency in Walloon Holstein cattle towards its current proceeding implementation. It will report complementary elements to submitted peer reviewed papers.

Materials and Methods

Used Data:

Phenotypic, pedigree and genomic data were acquired in collaboration with Elevéo (Awé groupe, Ciney, Belgium).

Methane Phenotypes:

Direct measurements of methane using respiration chambers, which are widely regarded as the gold standard, are costly, labor-intensive, and constrained by logistical challenges. Garnsworthy et al. (2019) compared chambers and various other direct methane measurement methods, noting that while each had its own strengths and limitations, all face significant barriers to really large-scale implementation. Breath sampling during milking and feeding (i.e., sniffers) was considered the one able to generate highest throughput but still needing high levels of maintenance of the installations that have to be distributed in many commercial farms. In contrast, mid-infrared spectrometry (MIR) is already used routinely in milk recording for phenotyping fat, protein and other milk components. Any novel predictions can be easily added as they exploit this existing infrastructure. Therefore, this enables low-cost, high-throughput phenotyping for CH_4 emissions, crucial for large-scale breeding programs. In order to differentiate from direct methane emission measurement MIR predicted methane emission will be called PCH4 (g/d).

Milk samples were collected between 2007 and 2023 during the official milk recording of Walloon Region of Belgium. The milk samples were analysed by MIR spectrometry (commercial instruments from FOSS) to generate MIR spectra. The milk spectra were standardized as described by Grelet et al. (2015). The development of MIR based PCH4 is an ongoing process. In this study the best equation developed by Vanlierde et al. (2021) with coefficient of determination (R^2), standard error, and root mean square error (RMSE) of cross-validation of 0.68, 57 g/d, and 58 g/d, respectively. The PCH4 records were extracted for Holstein cows divided into 3 traits according to parity: PCH41 for the first, PCH42 for the second, and PCH43 for the third parity. Records on DIM lower than 5 d and over 365 d were eliminated. The PCH4 records were limited to 100 to 800 g/d. In total, methane data (PCH4, g/d) on 285 530 first-parity (1 920 130 test-day records), 224 643 second-parity (1 516 843 test-day records), and 160 226 third-parity (1 072 725 test-day records) Holstein cows distributed in 1 520 herds in the Walloon region of Belgium were used. On average, 6.72, 6.75, and 6.70 test-day records were available per cow per parity.

Pedigree Data:
The cleaned pedigree from the Walloon genetic evaluation was used. Genetic groups were defined as in the evaluations by group of birth years, origin (Europe vs. USA) and sex. The pedigree used consisted of 439 214 animals, including 13 834 bulls.

Genomic Data:
Genomic data of 30 554 SNPs was available for 9 631 animals, including 1 823 bulls (either directly phenotyped or represented in the analysed pedigree) from the routine genetic evaluation system of Holstein cattle in the Walloon region of Belgium. After applying all quality control measures, non-mapped SNP, SNP located on sexual chromosomes, SNP with Mendelian conflicts, and those with minor allele frequency less than 5% were excluded. Finally, data of 28 513 SNPs located on 29 chromosomes were used.

Genomic Evaluation System:
A random regression test-day model (RR-TDM) was implemented, using the existing model for milk, fat, and protein yields as reference. As the used phenotype PCH4 is available at each test-day for each milk-recorded cow in the Walloon Region by direct substitution of milk, fat, protein by PCH4 as the target trait compatibility with established post-evaluation procedures was maintained, particularly for the estimation of reliability (REL). The model was applied jointly for first, second, and third lactations, treating each parity as a distinct trait.

Variance Component Estimation
Due to the large size of the dataset, variance components were estimated using a subset-based approach. Six random subsets were generated by sampling 10% of herds with replacement. Each subset was analyzed independently, with corresponding pedigree data extracted to include an average of 45 343 animals per subset. Variance components were estimated using the Expectation-Maximization Restricted Maximum Likelihood (EM-REML) algorithm. The final estimates for each component were obtained by averaging the results across the six subsets. Heritability was calculated daily across the lactation period and subsequently averaged. Genetic, permanent environmental, and herd-year variances were derived from the covariance matrices, while residual variances were modeled as trait-specific.

Genomic Evaluation and GEBV Computation
The RRTDM was solved as a ssGBLUP Model integrating pedigree relationship and genomic relationship matrix using an iterative on-data preconditioned conjugate gradient solver, enabling allowing efficient computation of genomic estimated breeding values (GEBV). Daily genetic random regression solutions were averaged over a standard 305-day lactation period for each of the three lactations to derive GEBVs.

Genomic Reliability (GREL) Estimation
Initial reliability estimates were computed using pedigree-based REL, following the same single-trait procedure used for traditional production traits. These REL values were then transformed into genomic reliability (GREL) using the methodology described by Gao et al. (2023) and Ben Zaabza et al. (2022). This transformation replaced the pedigree-based relationship matrix ($\mathbf{A}^{-1}$) with the genomic relationship matrix ($\mathbf{H}^{-1}$) for genotyped animals. allowing for improved accuracy and the propagation of genomic data to non-genotyped animals.

Integrating Methane in a Breeding Program:
There are several options to consider methane in a breeding program. Achieving a full integration in the breeding goal is currently limited by missing economic values—except in Denmark. A desired gain approach has also been considered, though it poses difficulties in optimization. We opted for a temporary solution, where animals would be ranked for

methane emissions while keeping productivity, functionality, or economic outcomes constant while maintaining breeding for increased economic performances. This approach leads to a residual-based efficiency trait, which can also be interpreted as correcting methane emissions for those specific performance levels. In this context, we tested three approaches:

1. Relative to production traits, leading to a Residual Methane Efficiency Production (RMEP) index;
2. Relative to functional traits, summarized in the Walloon V€F sub-index, leading to Residual Methane Efficiency Functional (RMEF);
3. Relative to all traits, using the Walloon V€G global index, leading to Residual Methane Efficiency Global (RMEG).

Higher values of RMEP, RMEF, and RMEG indicate more efficient animals. Therefore, these indices were expressed relative to all cows born in 2020 with records, standardized to have a mean of 100 and a standard deviation of 10.

Needed genetic parameters were estimated using 1,020 bulls, each meeting the following criteria: a minimum of 30 daughters phenotyped for PCH4, a reliability (REL or GREL) of at least 0.50 for PCH4 but also across all other 37 investigated traits or indices evaluated in our routine. Approximate genetic correlations were estimated based on birth year trend adjusted GEBV of the selected bulls using the procedure proposed by Blanchard et al. (1983).

Evaluating the Impact of Each Index:

The impact of the use of the RMEP, RMEF, and RMEG indexes was evaluated by plotting the PCH4 averages by daughter groups.

Comparing to other Genetic Evaluations:

Some other countries have started to produce GEBV for methane emissions based on breath measurements. However, public access to this information remains limited. We are reporting here only for two countries, one relying on sniffers and GreenFeed systems (Country A) and one using sniffers (Country B). Even if the available data was limited, and a few bulls have GEBV were reliable enough for meaningful comparisons. Despite this, this small study allowed them to compare our GEBV that are only milk composition based.

Results & Discussion

Descriptive Statistics and Genetic Parameters

Lactation curves of PCH4 for the first 3 parties are presented in Figure 1-A. The average daily PCH4 in the first parity was lower than in the second and third parities, ranging from 324 to 367 g/d. Estimated heritability (h^2) of PCH4 throughout lactation for the first three parities is presented in Figure 1-B. The results show that h² varies over lactation, peaking around DIM 200. The mean (SD) h^2 estimates for daily PCH4 were 0.23 (0.05), 0.21 (0.05), and 0.20 (0.05) in the first, second, and third parity, respectively. Figure 1-C presents the genetic correlations between lactations, visualized across the whole lactation. For a major part of the lactations the observed genetic correlations were very high, close to 1.

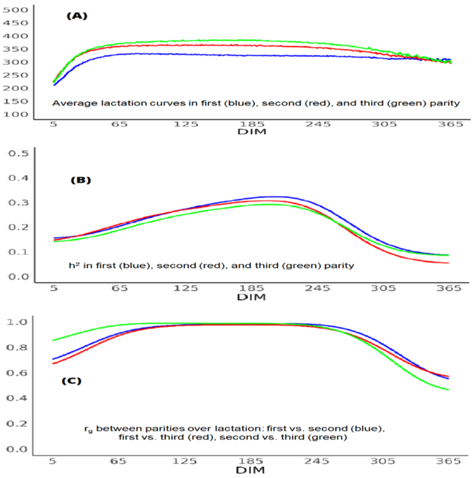

Figure 1. (A) Lactation curves for methane emissions (PCH4) in first (blue), second (red), and third (green) parity, (B) Heritability of PCH4 across lactation for each parity, and (C) genetic correlation across parities across the lactation.

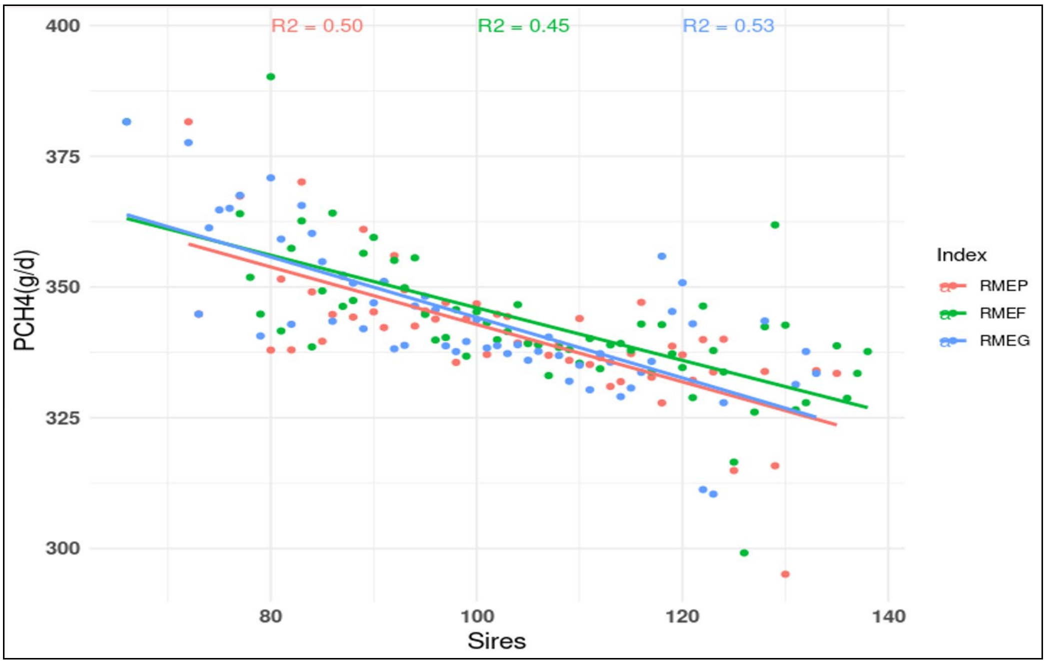

Figure 2. Distribution of average methane emissions (PCH4) for the 1020 daughter groups, sires sorted according to their relative GEBV, the consistency of the impact of selection being reported through R^2 values for RMEP, RMEF and RMEG.

When comparing the impact of selection base on the distribution of daughter groups for the different indexes (Figure 2), the RMEG showed the strongest impact.

For Country A, 382 of their published sires were in common to our 1020 animals. Most of these bulls were born in NLD (105), USA (103), CAN (57), DEU (46), ITA (23) and FRA (21). As illustrated in Figure 3, many of these bulls had low to very low reliabilities. This illustrates a common issue in methane phenotyping based on breath measurements, the scarcity of data.

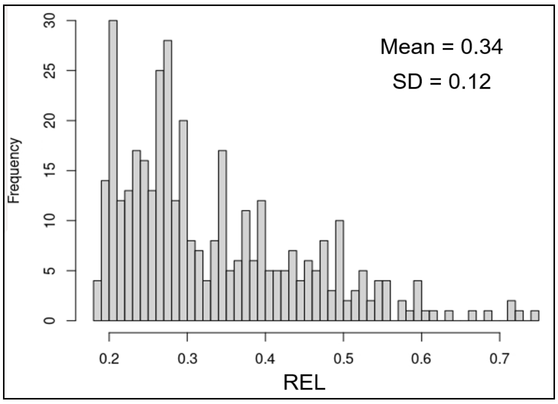

Figure 3. Distribution of reliability (REL) of common sires from Country A

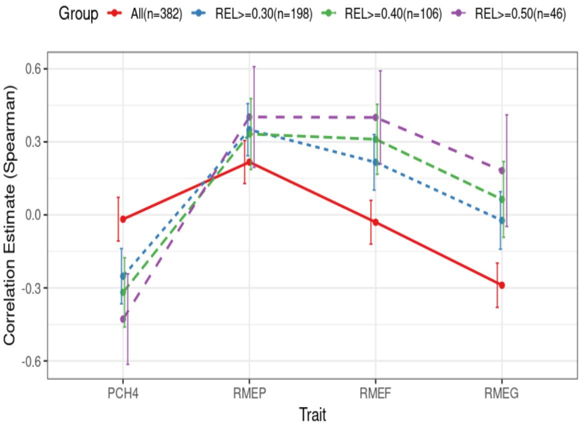

Figure 4. Visualization of the Blanchard et al. (1983) adjusted rank correlation based on common sires from Country A.

Figure 4 illustrates the rank correlations we observed with this population, based on different levels of foreign REL. Please note that we expressed PCH4 in its natural scale from low to high methane emissions, all other indexes, local or foreign, are defined from least to most desirable. After adjusting for the direction of correlation (SE) for the sires over REL of 0.50 were 0.41(0.19), 0.40 (0.21), 0.40 (0.19) and 0.17 (0.23) for PCH4, RMEP, RMEF and RMEG. These moderately positive values when comparing to a pure emission foreign EBV are encouraging that even totally different phenotyping strategies generate EBV that show the same tendency.

Figures 5 and 6 show similar figures but for Country B. However, only 14 sires were in common therefore the presented results should be considered in a very cautious manner.

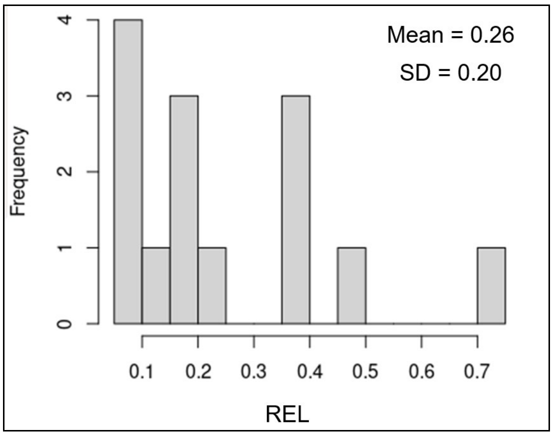

Figure 5. Distribution of reliability (REL) of common sires from Country B

As shown in Figure 5 the mean level of REL in the common bulls is extremely low.

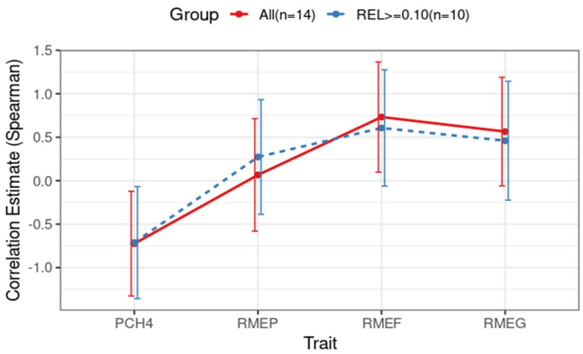

Figure 6. Visualization of the Blanchard et al. (1983) adjusted rank correlation based on common sires from Country B.

After adjusting for the direction of correlation (SE) for the sires over REL of 0.10 were 0.71(0.64), 0.27 (0.66), 0.61 (0.67) and 0.46 (0.68) for PCH4, RMEP, RMEF and RMEG (Figure 6). Even if these results are pure indication of a common trend, this trend is again positive, showing rather similar results.

Conclusions

We presented in this paper companion material shown at the INTERBULL Meeting 2025 to more detailed publications illustrating the novel genomic evaluation system for Methane Efficiency in Walloon Holstein cattle. Despite having a completely different approach to other countries which use sniffer and / or Greenfeed technology we estimated EBV that showed similar positive direction in terms of rank correlations.

We tested several residual-based efficiency indexes that could also be interpreted as correcting methane emissions for those specific performance as an interim solution for the integration of methane in breeding programs. The one relative to all currently selected traits, using the Walloon V€G global index, showed the most promising results and it the easiest to communicate. Indeed, it allows breeders to decide after selection the best animal based on V€G which one can be considered the most efficient.

Acknowledgments

The authors acknowledge the support of the whole Walloon Futurospectre Consortium including the University of Liège–Gembloux Agro-Bio Tech (Gembloux, Belgium), the Walloon Agricicultural Research Centre (CRA-W, Gembloux, Belgium), the Walloon Breeding Association (AWÉ Groupe, Elevéo, Ciney, Belgium) and the Walloon Milk Committee (Battice, Belgium). The authors are grateful to Jérémie Vandenplas (Wageningen University and Research) for his help when we calculated the genomic reliability. The ongoing support of the Walloon Government (Service Public de Wallonie – Direction Générale Opérationnelle Agriculture, Ressources Naturelles et Environnement, SPW-DGARNE, Namur, Belgium) through their support for the genetic evaluation systems, the ScorWelCow and WALLeSmart Projects (D65-1420 and D65-1435) and previous projects is also acknowledged. The University of Liège–Gembloux Agro-Bio Tech (Gembloux, Belgium) supported participation at the conference and computations through the technical platform Calcul et Modélisation Informatique (CAMI) of the TERRA Teaching and Research Centre, partly supported by the Fonds de la Recherche Scientifique–FNRS under grant no. T.0095.19 (PDR "DEEPSELECT"). Support by the Consortium for Computational Resources (CECI) of the Federation Wallonia-Brussels is acknowledged. The development of MIR-based equations was supported by different European projects and many international partners.

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Weighted single-step genome-wide association studies for methane intensity in Chinese Holstein cattle

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Abstract

Reducing methane emissions from dairy cows has been a key area of research in recent decades. This study aimed to identify genomic regions associated with methane intensity (MeI) in Chinese Holstein cattle. MeI phenotype was either predicted by mid-infrared spectra (MIRS, R2cv= 0.66) or directly measured by sniffer. Data were collected from eight commercial farms in Beijing between 2017–2020 and 2024. A weighted single-step genome-wide association study (WssGWAS) was performed based on 1,120 genotypes, 4,995 phenotypic records, and pedigree of 10 911 individuals.

The mean MeI was 7.67 ± 1.52 (g/kg milk yield). The estimated heritability of MeI was 0.15±0.04, and the repeatability was 0.42±0.02. Eleven 10-SNP windows harboring 19 protein encoding genes explained 2.17% of the genomic variance, with genomic regions on BTA1, 5, 8, 15, 19, 20, 24, 26, and 27. Five of the windows were also associated with milk production or milk component traits, while one window contained the QTL linked to metabolic body weight. The region explaining the highest proportion of variance (0.34%) was located on BTA15, which included five protein encoding genes. Among them, *SCN4B* and *MPZL3* are proposed as candidate genes.

In total, the preliminary results show that MeI is a heritable, repeatable, and polygenic trait in Chinese Holstein population. The identified MeI-related genomic regions provide an insight for breeding dairy cows with lower methane emissions.

Key words: dairy cattle, genetic parameter, WssGWAS, methane intensity

Introduction

Methane emissions from ruminants are a significant contributor to greenhouse gas emissions in agriculture. In China, approximately 24% of total methane emissions come from the production of livestock (Wang et al., 2024). In the past 30 years, the contribution of dairy cattle has notably increased, rising from 1.9% to 7% of the total emissions (Wang et al., 2024). Reducing methane emissions from cows is an issue that requires worldwide attention.

As we all know, animal breeding is a helpful method to reach this goal. To apply breeding techniques, large-scale recording of individual enteric methane emissions is essential (de Haas et al., 2017). However, methane emission is difficult to measure, and only few methods can costly generate large amount of data, such as sniffer and milk mid-infrared spectra (MIRS). With sniffer, individual cows can be recorded on a wide scale and at a reasonable cost (Garnsworthy et al., 2019). Using sniffers placed in the feed bin of automatic milking systems (AMS), this method measures the concentrations of gases. The present study also employed MIRS to predict the methane intensity of dairy cows. It is simple, high-throughput, and shows a great deal of potential for predicting methane emissions from dairy animals. The ability of MIRS to predict methane emissions has been widely reported (Coppa et al., 2022, Dehareng et al., 2012).

Among the various methane emission traits, the definition if methane intensity (MeI) is methane output relative to output such as milk production (de Haas et al., 2017). Specifically, MeI measures the amount of methane (CH_4) emitted per kilogram of output product, such as milk (g/kg), and is strongly influenced by both the milk production levels and the energy required for this process.

The main objective of this study is to 1) measure sniffer-based methane intensity and predict methane intensity based on MIRS in Chinese Holstein population; 2) estimate genetic parameters for methane intensity and, 3) identify candidate genomic regions for methane intensity.

Materials and Methods

Data and Sampling

Animals

Data were collected from July 2024 to November 2024 at two commercial farms in Beijing. A total of 208 cows were recorded during experiment.

Breath Sampling

All cows had access to an AMS (DeLaval International AB, Tumba, Sweden) for milking. Each barn was equipped with two AMS, but only one of them was installed with a sniffer. Cows were free to enter either AMS, with or without the sniffer (Guardian NG/Gascard, Edinburgh Instruments Ltd, Livingston, UK).

Data segments with no record of a cow entering the AMS within 5 minutes before or after were classified as ambient values. The ambient values recorded on a given day were averaged and used as the daily ambient mean.

While cows were inside the AMS, their heads could approach the gas collector positioned in the feed bin, as shown in Figure 1. Records of cows spending less than 2 minutes inside the AMS were excluded from the analysis. The raw data were preprocessed in four steps: (1) matching data from the AMS and sniffer to match a sniffer measurement with an identification number; (2) removing the first minute of each record; (3) using the 'findpeaks' function in R v4.3.2 to identify belching peaks. At least one peak must be found (exceed the mean ambient CH_4 concentration for the day by 200 ppm); (4) deleting consecutive when CO_2 concentration dropped below the lower 25% quartile of the mean CO_2 concentration for more than 10 seconds, indicating that the cow's head had left.

Figure 1. Gas collector in the feed bin

After processing, ambient-corrected gas concentrations for CH_4 and CO_2 were obtained by subtracting the ambient mean from the measured concentrations. The mean values of the gas concentrations and their ratio were calculated for each measurement. Subsequently, a three-step data quality control process was employed: (1) daily averages for the ambient-corrected gas concentrations were calculated after collecting all records for a single day. A twofold standard deviation quality control was used to eliminate records with excessively high or low gas contents; (2) records for measurement days with fewer than 10 cows were removed to avoid potential machine errors; (3) records with concentration ratios greater than the mean ± standard deviation of the concentration ratios for the same cow were removed.

Milk Yield, Body Weight, and Feed

For milk yield, the 3-day average was used as the daily milk yield (DMY). Milk composition data, including milk fat percentage, lactose percentage, and protein percentage, were collected from DHI. The closest DHI record to the methane measurement date (within 15 days) was selected for subsequent calculation.

Records with milk fat >7% or <2%, milk protein >5% or <2%, and daily milk yield <5 kg or >100 kg were excluded. Additionally, records with days in milk (DIM) <15 or >300 were removed. Energy-corrected milk was calculated using the formula from Sjaunja et al. (1990).

Body weight was expressed as weekly averages after a two-step quality control process: (1) cows whose body weight exceeded the upper or lower limits were removed (first parity: 450–750 kg; 2+ parities: 500–900 kg); (2) measurements within a single parity that differed by >50 kg from the mean were removed. After this, weekly averages of body weight were calculated. Since first-parity cows have greater weight variability, their weekly body weight average only represented the current week's weight. In contrast, body weight data from cows of later parities can represent the averages of the current, previous, and next week's body weight.

Feed data was provided by farm. Descriptive statistics of individual information, daily milk yield, body weight and diet crude fat for dairy cows is shown in Table 1.

Table 1: Descriptive statistics of individual information, daily milk yield, body weight and diet crude fat in Chinese Holstein cattle.

Trait	mean	SD	min	max
parity	2.46	1.33	1	7
days in milk	132.81	77.81	15	299
daily milk yield (kg)	42.85	9.03	17.14	66.48
body weight (kg)	690.53	87.81	481	888
diet crude fat (%DM)	5.58	0.42	4.91	6.42

Methane Intensity

Following the 'Model 2' developed by Kjeldsen et al. (2024), CO_2 production (CO_2P) was calculated. Subsequently, the methane and CO_2 concentrations from each measurement were averaged. Since the gases originated from the same breath, their concentrations were multiplied by their molecular weights before calculating the ratio to obtain the mass ratio (CH_4:CO_2). Methane intensity (MeI) was calculated as:

$$MeI = \frac{CH_4: CO_2 \times CO_2P}{DMY}$$

Given the variability in methane emissions at different times of day, a single measurement cannot accurately reflect an animal's true methane emission level. Therefore, weekly averages were used as the methane emission traits in this study. Weekly averages were calculated by retaining records from weeks with more than 4 measurements. Finally, 758 weekly averages were retained for subsequent analyses.

MIRS Prediction

Most of the milk spectral data were collected by the farm for DHI testing. In addition to the DHI sample collections, we also collected milk samples between two DHI samplings. All milk samples were analyzed using the same spectrometer (Banteley), which generates a spectrum of 899 wavelength transmittance values in the mid-infrared (MIR) region. The following spectral regions were retained for analysis, including 968.1–1 577.5 cm^{-1}, 1 731.8–1 762.6 cm^{-1}, 1 781.9–1 808.9 cm^{-1}, and 2 831.0–2 966.0 cm^{-1} followed Grelet et al. (2021), leaving a total of 215 wavenumbers. The spectra were preprocessed using Savitzky-Golay second-order derivatives, with spectral quality control conducted using pcout (Filzmoser et al., 2008). In addition to the MIRS data, individual information (parity, DIM, and DMY) were also included in the dataset for prediction. The data were processed to match a total of 227 records from 120 cows, which formed the training set (Dataset A).

Prediction Equation Development

Partial least squares regression (PLSR) was used to develop the prediction equation. Under 10-fold cross-validation, the model achieved an R^2 (coefficient of determination) of 0.66 and a Root mean square error of prediction (RMSE) of 1.25.

Then the prediction formula was employed in the dataset B (21 772 records with MIRS and individual information) to obtain phenotypes for a larger population. Dataset A was contained by dataset B. To ensure the usability of the prediction equations, the Mahalanobis distance (Mahalanobis, 1936) was calculated for MIRS in dataset B. Only data with Mahalanobis distance within that of dataset A were retained. Predictive equations for methane emission traits were built based on the training set and applied to dataset B for quality control of predicted methane emission phenotypes. When the records within the same individual parity was less than 3, all values for that parity were deleted. Subsequently, the coefficient of variation (CV) was calculated for each cow in single parity. Records with a CV greater than 25% were removed, leaving a total of 4 995 records from 1 187 cows.

Pedigree and Genotype

The pedigree of the cows with phenotypic records were traced back as many generations as possible. The final pedigree included 10 911 cows.

A total of 1,120 cows were genotyped using the Illumina 150K Bovine Bead Chip (Illumina Inc.). Genomic quality control was performed using PLINK v1.90 software (Purcell et al., 2007). Single nucleotide polymorphisms (SNPs) with minor allele frequencies lower than 0.1 or those with extreme deviations from Hardy–Weinberg equilibrium (P-value < 10^{-6}) were excluded. After quality control, a total of 109 619 SNPs were used in the study.

WssGWAS

The (co)variance components were estimated using AI-REML and EM-REML procedure implemented in the AIREMLF90 package from BLUPF90 (Misztal et al., 2014).

The variance components and genetic parameters was estimated based on the model:

$$y = X_1\beta + X_2\phi + Z\alpha + Wpe + e$$

y was the vectors of methane intensity. β was the vector of fixed effects for colostrum quality traits, including farm-season-year of calving (45 levels), parity (3 levels); ϕ was the regression coefficient of days in milk. α was the vector of random additive genetic effects, following $\alpha \sim N(0, H\sigma_a^2)$; pe was the permanent environment effect following $pe \sim N(0, I\sigma_{pe}^2)$; e was the vectors of random residual effects following $e \sim N(0, I\sigma_e^2)$; X_1, X_2, Z , and W, were the corresponding incidence matrices; H was the matrix of additive genetic relationships constructed from the pedigree and genotype; σ_a^2 was the additive genetic variance; I was an identity matrix, σ_{pe}^2 was the permanent environment variance, and σ_e^2 was the residual variance. The inverse of the H matrix (H^{-1}) was calculated as follows:

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

where A^{-1} is the inverse of the pedigree-based relationship matrix; A_{22}^{-1} is the A^{-1} for the genotyped animals; and $G^{-1}$is the inverse of the genomic relationship matrix. The G matrix was calculated according to(VanRaden, 2008):

$$G = \frac{ZDZ'}{2\sum_{i=1}^{M} P_i(1 - P_i)}$$

where Z is the matrix of genotypes adjusted for allele frequencies (0, 1, or 2 for aa, Aa, and AA, respectively); D is a diagonal matrix of weights for SNP variances (initially $D = I$); M is the number of SNPs, and P_i is the minor allele frequency of the i^{th} SNP.

The estimates of SNP effects and weights for the WssGWAS analyses (four iterations) for colostrum quality traits were obtained according to (Wang et al., 2014). The weight for each SNP was calculated as: $d_i = 1.125^{\frac{|\hat{a}_i|}{sd(\hat{a}_i)} - 2}$ (VanRaden, 2008), where i is the i^{th} SNP. The percentage of the total addictive genetic variance explained by the i^{th} region was calculated as:

$$\frac{Var(a_i)}{\sigma_a^2} \times 100\% = \frac{Var\left(\sum_{j=1}^{10} Z_j \hat{u}_j\right)}{\sigma_a^2} \times 100\%$$

where a_i is genetic value of the i^{th} region that consists of contiguous 10 SNPs, σ_a^2 is the total additive genetic variance, Z_j is a vector of gene content of the j^{th} SNP for all individuals, and $\hat{u}_j$ is the marker effect of the jth SNP within the i^{th} region.

Non-overlapping contiguous genomic windows that explained 0.15% or more of the total additive genetic variance were considered to be associated with the trait. Candidate genes were identified by examining genomic windows based on the ARS-UCD1.2. The biological functions of these genes, Gene Ontology (GO) terms (Ashburner et al., 2000) enrichment were identified using the R package "BiomaRt" (Durinck et al., 2009) and "clusterProfiler" (Wu et al., 2021). The genomic regions were compared to cattle QTL database (Hu et al., 2022).

Results & Discussion

As presented in Table 2, the average MeI was 7.22 ± 1.99 g/kg in the current population. The predicted methane intensity closely followed the observed values, with a predicted MeI of 7.67 ± 1.52 g/kg.

Table 2: Descriptive statistics of methane intensity (MeI) and predicted methane intensity (PMeI) in Chinese Holstein cattle.

Trait	mean	SD	min	max
MeI (g/kg)	7.22	1.99	3.11	15.04
PMeI (g/kg)	7.67	1.52	3.14	13.62

Different MeI values have been recorded in earlier studies. In a mixed cow herd, MeI ranged from 3.0 to 36.0 g/kg, with an average of 13.5 ± 3.92 g/kg reported by Niu et al. (2018). Similarly, in a population of French Holstein cattle, Fresco et al. (2023) reported a MeI of 11.7 ± 2.6 g/kg. In this study, MeI was lower than those found in these studies, but it was closer to 8.61 ± 1.15 g/kg in dairy cattle reported by Lassen and Løvendahl's (2016).

PMeI showed moderate heritability according to our research. The results indicate that the heritability estimate for PMeI was 0.15±0.04 and the repeatability was 0.42±0.02. In previous study, MeI or PMeI heritability ranged from 0.04 to 0.35. In a population of 1 091 Swiss Brown cows, Bittante and Cecchinato et al. (2020) observed a heritability of 0.12 ± 0.06, which is similar to our study. While Fresco et al. (2024) reported a heritability of 0.35 ± 0.04 using a very large dataset (n = 167 514), Lassen and Løvendahl (2016) estimated a heritability of 0.21 ± 0.06 using a population of 3 121 cows. Higher heritability values than those obtained in our study were found in both of these studies. However, our result was lower such as the heritability of 0.04 ± 0.03 estimated by Manzanilla-Pech et al. (2022) using of 1 962 Danish Holstein cows. The breed, gas measurement techniques and equipment, and raising conditions of dairy cows are some of the variables that affect the heritability estimate of MeI or PMeI in various populations. The heritability estimates in the current population are in the medium range when compared to the findings of other studies.

In this study, we identified eleven genomic regions on *Bos taurus autosome* (BTA) 1, 5, 8, 15, 19, 20, 24, 26, and 27 that explained more than 0.15% of the genetic variance as Figure 2. These regions, which harbor a total of 19 protein-coding genes, accounted for 2.17% of the genomic variance. The window that explained the highest genetic variance was located on BTA15, which explained 0.34% of additive genetic variance and contained five genes, including *JAML*, *SCN2B*, *TMPRSS4*, *SCN4B*, and *MPZL3*. Two of these genes were enriched by the significant GO terms, which were *SCN2B* and *MPZL3*.

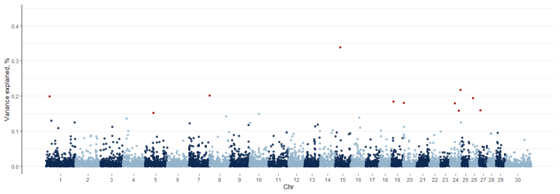

Figure 2. Proportion of the total additive genetic variance of 10-SNP genomic windows based on the weighted single-step genome association study for predicted methane intensity. Red points represent the windows exceed the 0.15% threshold of the total additive genetic variance.

Table 3: Quantitative trait loci reported for *Bos taurus* associated with genomic regions that explained more than 0.15% of the additive genetic variance for predicted Methane intensity

Chr	Regions (Mb)	Explained genetic variance, %	Associated trait
1	20.03-20.29	0.20	MP
5	44.96-45.20	0.15	MF, MY
24	47.10-47.32	0.16	MF
24	56.77-56.93	0.22	MP, BW
26	19.74-20.23	0.19	MF, MP

MP: milk protein, MF: milk fat, MY: milk yield, BW: body weight

Additionally, we referred to the Cattle QTL database to examine potential QTL overlaps with genomic regions that explained more than 0.15% of the additive genetic variance. Table 3 shows five genomic regions containing QTLs associated with milk protein, milk fat, milk yield, and body weight. However, the relationships of MeI with these traits still needs to be further explored.

Conclusions

Methane intensity can be measured and predicted by milk mid-infrared spectra. It is a moderate heritable, polygenic trait in Chinese Holstein population. However, these are relatively preliminary findings, and further research is still necessary.

Acknowledgments

Thanks for Project of National Key R&D Program of China (2022YFE0115700), and all the support from Team 459 in China Agricultural University, Aarhus University, Beijing Sunlon Livestock.

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Genetic evaluation for longevity of dairy cattle in the Netherlands

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Abstract

Longevity of dairy cattle is an important trait from an economic and welfare perspective, as well as from a societal and government perspective. For a farmer it is beneficial to keep older cows, as it will reduce costs of rearing. The Dutch government aims to reduce the environmental impact of livestock, and for that it is also beneficial to keep older cows. Older cows produce more on average, and feed is converted more efficiently. In the Dutch-Flemish genetic evaluation of functional longevity a random regression animal model with a fifth order Legendre polynomial is used, where within-herd production level is fitted as an explanatory effect. Published breeding values for longevity are partially based on predictor traits. The breeding values for milk production, as well as udder health, claw health and locomotion are added as predictor traits through selection index theory. In this paper the impact of the introduction of genetic evaluation of longevity is assessed. Over the past 25 years all statistics on longevity metrics have been favorable. Productive life increased with 337 days to 1,445 days for cows culled in 2024. Together with a reduced amount of youngstock, the rearing period reduced 40 days to 763 days. The mean number of calvings increased by 0.8 to 3.9 calvings. Lifetime production increased in 25 years by 14,329 kg to 38,283 kg of milk (with 4.40% fat and 3.58% protein), resulting in 1,684 kg fat and 1,369 kg protein. Production per day of life increased by 4.8 kg to 17.1 kg of milk. Longevity is a result of management (e.g. feeding, housing and culling decisions), environment, and genetics. Genetically, longevity increased by 600 days, which suggests that the full genetic potential is not yet utilized. The strong increase of the genetic trend for longevity was supported by selection on udder health, claw health and feet & legs. Culling decisions can also be affected by governmental changes in regulations. The genetic trend is more consistent over the years than the phenotypic trend. Long-term trends show that all these factors together resulted in significant improvements for longevity and lifetime production. With the continuing improvement of production and health traits further improvement of longevity is expected.

Key words: longevity, genetic evaluation, trends

Introduction

Longevity of dairy cattle is an important trait from an economic and welfare perspective, as well as from a societal and government perspective. For a farmer it is beneficial to keep older cows, as it will reduce costs of rearing. The Dutch government aims to reduce the environmental impact of livestock, and for that it is also beneficial to keep older cows. Older cows produce more on average, and feed is converted more efficiently.

The genetic evaluation for longevity was introduced in 1999 based on a proportional hazard model and the published breeding value was for functional longevity, where functional longevity is longevity corrected for within-herd production level. The published breeding value changed in 2008 to true longevity, meaning that the adjustment for production was removed from the statistical model. In 2018 the statistical model was revised and changed to a random regression animal model, and true longevity is published.

Since the introduction of the breeding value of longevity it has not been investigated how lifetime performance of Dutch and Flemish cattle has evolved, and in this paper the impact of genetics on lifetime performance is assessed.

Genetic evaluation

Data

Length of productive life is defined as the time from first calving to the last test date for milk production, before the animal died or was culled for slaughter; this also included dry periods. The analysed period is length of productive life until 72 mo after first calving. The data set is constructed from records of pedigree, lactations and movements of cows in the Netherlands and Flanders. Herdbook-registered cows from a dairy breed with a test-day record on or after January 1, 1988 are included for Dutch data, and on or after January 1, 2006 for Flemish data. Data up to February 14, 2025 are included in the most recent genetic evaluation of April 2025. Cows are required to have an age at first calving between 20 and 40 mo. If the first calving of a cow took place before the starting date of the study, the record is considered to be left-truncated. Records of cows that are still alive at the time of data collection are considered to be right-censored. Records of cows that moved to another milking herd are also considered to be right-censored, if this herd is not participating in a milk recording scheme.

Records are constructed for each month a cow is present in a herd, from first calving up to the month the cow is culled, or 72 mo, or when the cow is censored. A cow culled in month *j* has *j* – 1 records with score 100 (alive), and record j with score 0 (culled). Monthly records are treated as missing after culling.

Additional selection criteria included: 1) data of a cow is used after a waiting period of 120 days after first calving; 2) Culled heifers without a milk testing, mostly culled before the first milk test, present in a herd with milk recording, are included; 3) Herd-year-months need to have a survival rate of at least 70%; 4) Herd-year-months with 5 or more culled animals need to have a survival rate that is higher than the mean survival rate of the past 12 months minus three times the standard deviation of survival of the past 12 months.

Statistical model

The genetic evaluation for the Netherlands and Flanders is a random regression animal model where survival per month is analysed:

$$\begin{aligned} Y_{ijklmno} = {} & HYS\_LS_i + YSAM\_LS_j + HSC_k \\ & + het_l + rec_m \\ & + \sum_{q=0}^{5} animal_{nq}\, leg_{oq} \\ & + error_{ijklmno} \end{aligned}$$

where

$Y_{ijklmno}$: observation for survival in month *o* after first calving; mo 1 – 72;

HYS_LS_i : fixed effect for herd-year-season x lactation-stage *i*; year-season observation, lactation split in 1, 2, 3+, stage of lactation split in mo 1-2, 3-9, 10+ and dry period;

$YSAM\_LS_j$: fixed effect for year-season x AFC x within-herd production level x lactation-stage *j*; year-season of observation, AFC in months 20, 21, …, 34, 35+, within-herd production level is defined per 3 years and is divided in 5 classes of 20% each for predicted or realised age-corrected 305-day yield of kg fat and protein;

HSC_k : fixed effect for herd size change *k*; HSC is calculated by comparing the number of cows present in a herd in a year with the number of cows in the same herd one year later. Seven classes are distinguished: shrinkage between 90 and 50%, shrinkage between 50 and 30%, shrinkage between 30 and 10%, neither shrinkage nor growth over 10%, growth between 10 and 30%, growth over 30%, and herds that were terminated (more than 90% shrinkage).

het_l : covariable for heterosis *l* of animal *n*;

rec_m : covariable for recombination *m* of animal *n*;

$animal_{nq}$: additive genetic random regression coefficient of animal *n* corresponding to polynomial *q*;

leg_{oq} : covariates of order q Legendre polynomial for month o;

$rest_{ijklmno}$: random residual effect of $Y_{ijklmno}$.

Within-herd production level is fitted to correct for culling due to low production, which is assumed to be the major source of voluntary culling yielding EBV for functional longevity.

Breeding values

In the direct breeding value estimation breeding values for functional longevity are estimated, because survival per month is adjusted for within-herd production level in the statistical model. Bull owners and farmers are used to using the breeding value for true longevity. With a selection index true longevity is derived from functional longevity and the production traits kg milk, kg fat, and kg protein.

Indirect information is used next to the direct information for longevity to increase the reliability of the breeding value longevity for young animals, as little direct information is available. Traits that are early available in life are preferred to increase the reliability, and for this the breeding values for subclinical mastitis, claw health and locomotion are used in a selection index.

Results & Discussion

The total data set for the routine genetic evaluation of longevity of April 2025 comprised 481,058,418 records from 14,292,149 animals in 44,328 herds. The pedigree included 16,834,548 animals including 226 phantom groups.

Genetic trend

The genetic trend for longevity for black & white Holstein cows is shown in figure 1. Since 2000 the genetic level increased by 615 days. Up to 2010 the average increase per year was 17 days, and since 2010 the increase per year increased to 37 days.

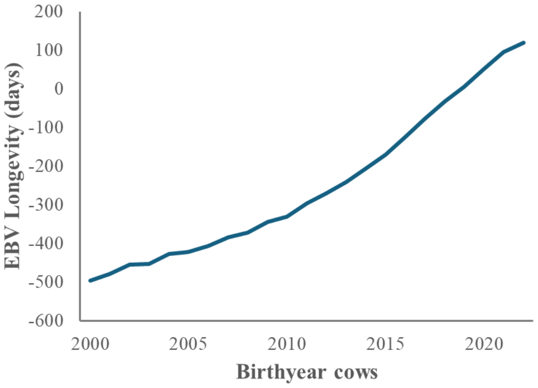

Figure 1. Genetic trend for black and white Holstein cows in the Netherlands.

Phenotypic trend

The phenotypic trend for productive life for culled herdbook cows in the Netherlands is shown in figure 2. From 2000 to 2024 the productive life increased by 337 days from 1,108 days to 1,445 days. This increase fluctuates over these 25 years. From 2000 to 2008 every year there was an increase in productive life. After 2008 productive life stabilized until 2016. From 2017 to 2019 the productive life declined because of culling excess cows related to national phosphate regulation. Since 2019 the productive life shows a sharp increase of 200 days in just four years. In the last two years, 2023 and 2024, the increase in productive life leveled off, likely due to more culling due to blue tongue infections.

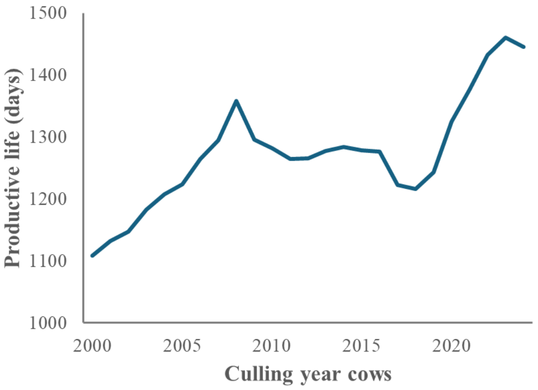

Figure 2. Phenotypic trend for productive life for culled herdbook cows in the Netherlands.

Genetic vs. phenotypic trend

The genetic trend and phenotypic trend both showed an increase since 2000. However, the phenotypic progress was 337 days, and the genetic progress was 615 days. Genetically the cows have the potential to get older than what is currently achieved.

The genetic trend for longevity shows a steady yearly increase with an acceleration since 2010 due to the introduction of genomics. The phenotypic trend shows more fluctuations, where especially the decline in the years 2017 to 2019 showed that national regulations can have significant effect. Also, the increase in productive leveled off in 2023 and 2024 showed that the environment in the form of disease pressure (blue tongue infections) has a marked effect.

The sharp increase in productive life since 2019 is the result of a change in replacement strategy of farmers. The incentive in change in replacement strategy is that it is more profitable to have a larger proportion of dairy cows compared to replacements. Genetically the cows are able to produce longer, and the sharp increase in productive life showed that cows are able to show their genetic potential.

Figure 3 shows the realized extra days for productive life for the daughters of black & white Holstein bulls born in 2012. The bulls are divided in four EBV classes. The daughters of the bulls with a breeding value longevity between -250 and 0 days had 1266 productive days after their first calving. Compared to the lowest EBV class these daughters have on average 112 days longer productive life. The daughters of bulls in the highest EBV class had 1484 productive days. The achieved productive life corresponds well with the breeding value of the EBV class. The difference between the productive life of the daughters of the best and the lowest scoring bulls for longevity is 330 days, and the difference in EBV is with 719 days. The expected difference is half of the EBV when mated on average cows, and the phenotypic difference is close to half of the genetic difference.

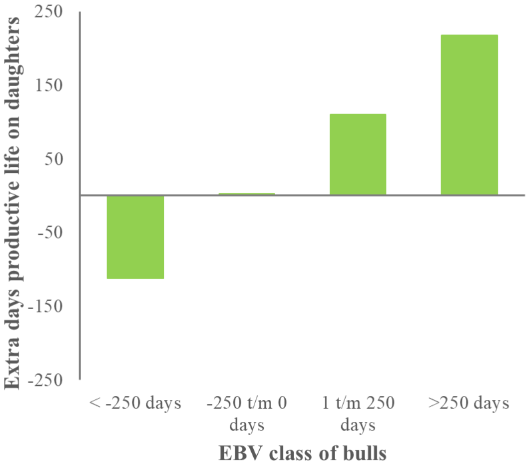

Figure 3. Realised extra days for productive life for daughters of black & white Holstein bulls born in 2012 divided in four EBV classes.

Change over time

Previous research showed that longevity changed over time. Van Pelt *et al.* (2016b) showed that the culling for low production reduced phenotypically. To compare animals genetically over time it is preferred to account for within-herd production level (Van Pelt *et al.*, 2016a), as genetically functional longevity showed less or no bias over years. For this reason, the genetic evaluation is analysing functional longevity.

The genetic parameters used in the genetic evaluation are based on phenotypic data from 1988 up to 2015. From the parameters genetic part-whole correlations can be derived as described in Van Pelt *et al.* (2015). The genetic parameters were re-estimated on a more recent data set with phenotypic data from 2008 up to 2023. In figure 4 the part-whole correlations for functional longevity are shown for the current parameters and the re-estimated parameters. With the current parameters the genetic correlation of cumulative survival up to 6 months after first calving with 72 months after first calving is 0.81, and then gradually increases. This shows that survival in early life is genetically different from survival later in life. With the re-estimated parameters based on more recent years the genetic correlations up to 24 months after first calving with total survival up to 72 months are lower than with the current parameters, and as low as 0.75. Over the years, survival in early life is genetically more different from survival in later life.

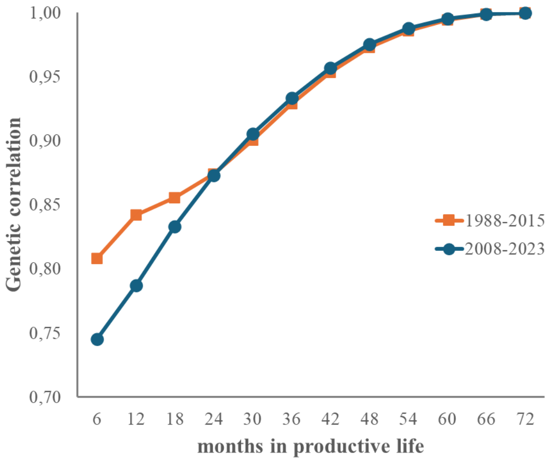

Figure 4. Genetic part-whole correlations for functional longevity based on phenotypic data from 1988 up to 2015 and from 2008 up to 2023.

Lifetime performance statistics

Over the past 25 years all statistics (CRV, 2024) on longevity metrics have been favorable (Table 1). Productive life increased with 337 days to 1,445 days for cows culled in 2024. Together with a reduced amount of youngstock, the rearing period reduced 40 days to 763 days. The mean number of calvings increased by 0.8 to 3.9 calvings. Lifetime production increased in 25 years by 14,329 kg to 38,283 kg of milk (with 4.40% fat and 3.58% protein), resulting in 1,684 kg fat and 1,369 kg protein. Production per day of life increased by 4.8 kg to 17.1 kg of milk. The highest relative change of 61% was achieved for lifetime production kg fat + protein. This is a result of the underlying traits; the rearing period reduced (-5%), production days increased (+34%), and kg m/day increased (+19%).

Longevity is a result of management (e.g. feeding, housing and culling decisions), environment, and genetics, as shown by the genetic and phenotypic trends. The genetic trend is also a result of the genetic response achieved by selecting on the Dutch/Flemish total merit index NVI.

Table 1. Lifetime performance statistics of culled herdbook cows in the Netherlands in 2000 and 2024.

	Culling year		Change	
	2000	2024	Abs.	Rel.
Calvings (nr)	3.1	3.9	0.8	26%
Production days	967	1,291	324	34%
Herdlife (d)	1,957	2,238	281	14%
kg m/day[1]	24.9	29.7	4.8	19%
LTP kg f+p[2,3]	1,895	3,053	1,158	61%
LTP kg m	24,044	38,283	14,239	58%
Productive life(d)	1,108	1,445	337	30%
Rearing period(d)	803	763	-40	-5%
kg m/day of life	12.3	17.1	4.8	39%
kg f+p/day of life	0.97	1.36	0.40	41%

[1]kg m : kg milk, [2]LTP: lifetime production, [3]kg f+p : kg fat + protein

The breeding goal has evolved over time from only including production traits, followed by including and put more emphasis on longevity and health traits. All traits in the NVI have favorable genetic correlations with longevity, resulting in the highest genetic response for longevity from all breeding goal traits.

Conclusions

Phenotypically, longevity increased by 337 days since 2000. Genetically, longevity increased by 600 days, which suggests that the full genetic potential is not yet utilized. The strong increase of the genetic trend for longevity was supported by selection on udder health, claw health and feet & legs. Culling decisions can also be affected by governmental changes in regulations. The genetic trend is more consistent over the years than the phenotypic trend. Long-term trends show that all these factors together resulted in significant improvements for longevity and lifetime production. With the continuing improvement of production and health traits further improvement of longevity is expected.

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Modernizing Canada's Lifetime Performance Index (LPI)

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Abstract

National selection indexes combining important traits are frequently used by dairy farmers, breeders, and A.I. companies to achieve their breeding goals. The Canadian dairy industry has made significant genetic progress with two national selection indexes, the Lifetime Performance Index (LPI) and Pro$, which are now double the rate compared to the pre-genomics era. Since its introduction in 1991 the LPI formula has changed alongside the expansion of national breeding objectives. With the introduction in recent years of genetic evaluations for its portfolio of traits related to sustainability, the timing was right for Lactanet to modernize several aspects of Canada's LPI, effective April 2025. A key strategic change is the creation of six subindexes, which are each published on their own using a standardized scale with an average of 500 and standard deviation of 100. Subindexes include the Production Index (PI), Longevity & Type Index (LTI), Health & Welfare Index (HWI), Reproduction Index (RI), Milkability Index (MI), and Environmental Impact Index (EI). For the Holstein breed, the relative weights placed on these subindexes are 40% PI, 32% LTI, 8% HWI, 10% RI, 5% MI, and 5% EI. The six other dairy breeds evaluated have differing relative weights in accordance with the respective breed objectives. A second important change is an increased focus on presenting the genetic response over the next five years that can be expected for each trait based on the average level of selection gain realized for LPI. While this approach recognizes the impact of direct inclusion of a trait in one of the six LPI subindexes, it also reflects the expected response for correlated traits. Defining six subindexes that contribute to LPI demonstrates the increased diversity of traits currently evaluated and acknowledges the continued expansion of Canada's overall breeding goal for dairy cattle breeds.

Key words: Lifetime Performance Index, subindexes, expected response

Introduction

Given the vast number of traits evaluated in dairy cattle breeding, most countries use at least one national genetic selection index to identify superior males and females in each breed. In Canada, the Lifetime Performance Index (LPI) was introduced in 1991 as the official ranking index for all seven dairy breeds, namely Ayrshire, Brown Swiss, Canadienne, Guernsey, Holstein, Jersey and Milking Shorthorn. A second national genetic selection index, named Pro$, was introduced in 2015 (Van Doormaal et al., 2015). With the relatively small population size of the Brown Swiss, Canadienne, Guernsey and Milking Shorthorn breeds in Canada, combined with the very high correlation (i.e.: over 85%) between the two national indexes, Pro$ values are only published for the three other breeds, Ayrshire, Holstein and Jersey.

Since the introduction of LPI in 1991, the traits included, and their relative weights, were previously reviewed and modified seven times with the latest being in 2019. While the original LPI formula included only production (60%) and type (40%) traits, herd life and somatic cell score were included in 2001, and a Health & Fertility component was first introduced with the addition of daughter fertility in 2005. The formula changes in 2008, 2015 and 2019 all increased the relative emphasis on the Health & Fertility component compared to the Production and Durability components, which have been 20:40:40, respectively, since 2019.

Canada was among the first countries globally to introduce national genetic evaluations for Feed Efficiency in 2021 (Jamrozik et al. 2021), which was then included in the Holstein LPI formula as an add-on trait starting in April 2022. In April 2023, two more traits related to environmental sustainability were launched by Lactanet Canada. Body weight data, converted to metabolic body weight, is used as the input phenotype to produce single-step genetic evaluations for Body Maintenance Requirements (Fleming et al., 2023). Together with Feed Efficiency, selection aims to reduce on-farm feed costs. Lactanet Canada was the first country to use milk mid-infrared (MIR) spectral predictions of methane yield as input phenotypes for its single-step genetic evaluation for Methane Efficiency (Van Doormaal et al., 2023; Oliveira et al., 2024). With this portfolio of traits available to help farmers genetically select to reduce the carbon footprint of their herd, the timing was right to modernize the LPI formula to allow for the inclusion of traits related to environmental sustainability.

There were multiple other goals underlying the need to modernize Canada's LPI formula, including:

- Reduce the mathematical nature of the formula and how to communicate it to breeders.
- Replace the three LPI components with six subindexes to be published on their own as well as be combined into LPI.
- Enhance the breeder understanding and language towards expected response by trait from index-based selection, instead of focusing on the specific traits included in the index and their relative weights.

Materials and Methods

Correlation Matrix and Expected Response

Official genetic evaluations for bulls within each breed served as the basis for the analysis of correlations and expected selection response. For each breed, progeny proven sires included in the genetic base definition for each breed were combined with younger genomic bulls with at least 30 registered daughters in Canada but not progeny proven for production and type traits. A matrix of simple correlations among all traits and indexes was calculated based on the official genetic evaluations published for the group of bulls included for each breed.

As described by Van Doormaal et al. (2015) for the development of Canada's profit-based national selection index, Pro$, correlations between any given index and individual traits can be used to estimate the expected response for the trait resulting from selection for the given index. Technically speaking, this requires true genetic correlations, but the use of bull evaluation correlations leads to easier calculations and serves as an excellent proxy. Such expected selection responses are more relevant than the traditional use of relative weights on traits included in an index, which often ignore the underlying correlation matrix among all traits.

To facilitate dairy farmer understanding of the concept of expected selection response, the bull evaluation trait correlations with the LPI national selection index were converted to units of expected genetic gain in the next five years. To accomplish this, the total genetic gain realized in Canadian cows and heifers born in the most recent 5-year period was calculated and then expressed in terms of standardized units based on the standard deviation of LPI values for Canadian females. For each individual trait, the 5-year expected response from selection for LPI was estimated by multiplying the total realized standardized gain for LPI by the trait correlation with LPI and the trait standard deviation based on published cow evaluations.

Formulation of LPI Subindexes

Since 2005, the Canadian LPI formula has included three components: Production, Durability and Health & Fertility. A key goal of the new modernized LPI formula was the development of six subindexes to better reflect the diverse groups of traits currently in Canada:

- Production Index (PI)
- Longevity & Type Index (LTI)
- Health & Welfare Index (HWI)
- Reproduction Index (RI)
- Milkability Index (MI)
- Environmental Impact Index (EI)

For the development of each subindex by breed, consultations with each breed association were held to identify any specific traits for which they desired targeted gains for the future. For the Production and the Longevity & Type subindexes, breed associations and industry organizations agreed to implement only minor modifications. In addition to separating the former Health & Fertility component into the new Health & Welfare and Reproduction subindexes, there was also a desire to include new traits in each subindex based on the underlying correlation matrix.

Given the increasing adoption of robotic milking systems in Canadian herds, which now represents one-quarter of milk-recorded cows, the industry agreed to develop a new Milkability subindex of LPI. In addition, with the launch of three new traits aimed at reducing the carbon footprint of dairy farms, a new Environmental Impact subindex was developed and included in the LPI for Holsteins.

Results & Discussion

Following industry consultation including the comparison of results from various LPI scenarios, Table 1 represents the final relative weight on each of the six subindexes in the modernized LPI for the Holstein breed, as well as the resulting correlation between each subindex and LPI. Similar relative weights were used for building the LPI for the six other dairy cattle breeds evaluated by Lactanet but are not presented here.

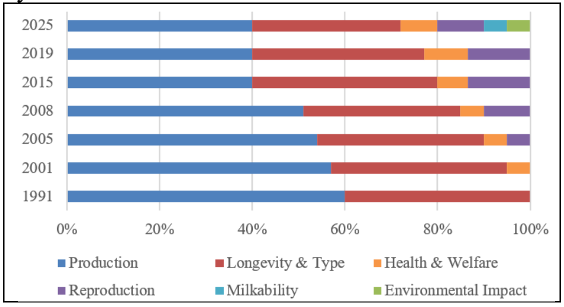
Table 1: Relative weight (%) of each subindex of the modernized LPI for Holsteins and their resulting correlation with LPI.

		%	**LPI Corr**
Production	**PI**	**40**	**0.83**
Longevity & Type	**LTI**	**32**	**0.69**
Health & Welfare	**HWI**	**8**	**0.57**
Reproduction	**RI**	**10**	**0.39**
Milkability	**MI**	**5**	**0.01**
Environmental Impact	**EI**	**5**	**0.00**

The Production Index has the highest relative weight of 40%, which also yields the highest correlation with LPI of 0.83. The Longevity & Type Index represents 32% of the LPI formula and has a relatively strong correlation of 0.69 with LPI. Results for the Health & Welfare and Reproduction Indexes are of particular interest since their relative weights of 8% and 10%, respectively, result in LPI correlations of 0.57 and 0.39. The lower LPI correlation for Reproduction stems from the underlying negative correlation between female fertility traits and other key traits in the LPI, especially milk yield and some key conformation traits. Both the Milkability and Environmental Impact Indexes are new subindexes of the LPI formula. Based on discussions with industry organizations, a relative weight of 5% on each was implemented. As shown in Table 1, these weights yield a correlation with LPI that is near zero at 0.01 and 0.00, respectively. In both cases, exclusion of these new subindexes of LPI would have yielded a negative correlation and expected selection response, which is not desired. In addition, for the Environment Impact Index the relative weight of 5% serves as a starting point to raise dairy farmer and industry awareness for the opportunity to genetically select for the underlying traits related to the carbon footprint of a dairy herd, even while there is no direct financial incentive to do so at the present time.

The 2025 update to the LPI formula represents the sixth significant modification since its inception in 1991. Figure 1 presents the evolution of traits that have been included by presenting them in groups aligned with the six subindexes included in the 2025 LPI formula for Holsteins.

Figure 1: Evolution of the Holstein LPI formula expressed as relative weights (%) on traits grouped by the six subindexes of the 2025 modernized LPI.



In the 1990s, the LPI formula only included production and type, with relative weights of 60:40, respectively. Herd Life and Somatic Cell Score were added in 2001. Daughter Fertility was added in 2005 and then increased in emphasis in 2008. The updates in 2015 and 2019 included higher relative weights on traits related to the current Health & Welfare and Reproduction subindexes, which therefore decreased the relative emphasis placed on the other subindexes, including production traits. Since 2015, however, the Production Index has maintained a 40% weight in the Holstein LPI, even with the addition of the new Milkability and Environmental Impact subindexes in 2025. The 2025 focus on estimates of expected response by trait resulting from LPI selection, rather than on traits included and their relative weights, slightly reduced the overall weight on traits related to the Health & Welfare and Reproduction subindexes (Figure 1).

Production Index (PI)

Figure 2 shows Fat and Protein Yields as the only two traits directly included in the Production Index, with relative weights of 60% and 40%, respectively. The inclusion of only these two traits also applies for all other breeds. When selecting for LPI, however, five other traits related to the Production subindex are monitored as correlated traits, namely Milk Yield, Fat and Protein Deviations and Lactation Persistency.

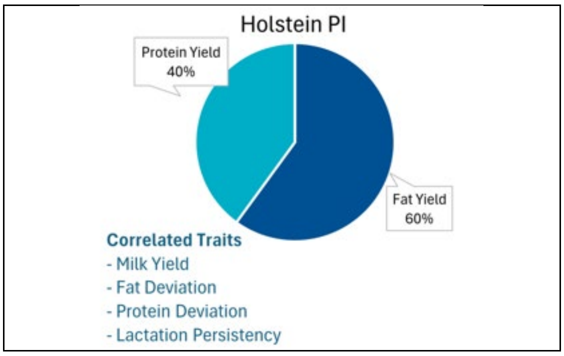


Figure 2. Traits included in the Production Index (PI) of LPI for Holsteins, with their relative weights, and key correlated traits.

In addition to the trait weights for the Production Index, Table 2 provides the resulting correlation that the direct and correlated traits have with LPI, given the relative weight of all six subindexes in Table 1. These correlations are also expressed in terms of the expected selection response (ESR) for each trait based on selection for LPI over the next five years.

Table 2: Relative weight (%) of traits included in the Production Index (PI) of LPI for Holsteins, their resulting correlation with LPI, and the expected selection response (ESR) by trait over the next 5 years resulting from selection for LPI.

	%	LPI Corr	ESR
Milk Yield		**0.43**	**534**
Fat Yield	60	**0.81**	**42.1**
Protein Yield	40	**0.74**	**28.0**
Fat Deviation		**0.56**	**0.29**
Protein Deviation		**0.54**	**0.13**
Lactation Persistency		**0.10**	**0.5**

The results in Table 2 clearly demonstrate the importance of concentrating on expected response versus trait emphasis in any index. For example, although Milk Yield has no direct weight in the Production Index, selection for LPI is still expected to result in over 500 kg of genetic gain for milk production in the Canadian Holstein population over the next five years, in addition to 42.1 kg and 28.0 kg for Fat and Protein Yields, respectively. In a similar manner, selection for the yields of milk components, without any direct weight on milk production also results in relatively strong expected gains for Fat and Protein Deviations (Table 2). Without direct inclusion of Lactation Persistency in the Production Index for Holsteins, the resulting correlation with LPI of 0.10 translates to little expected selection response but the ESR value is in the desired direction.

Longevity & Type Index (LTI)

The main goal of the Longevity & Type Index is to provide a subindex that allows dairy farmers the opportunity to select for increased longevity and functional conformation. For this reason, Mammary System, Feet & Legs and Herd Life have the highest direct emphasis in this subindex, with relative weights of 37% 33% and 20%, respectively (Figure 3).

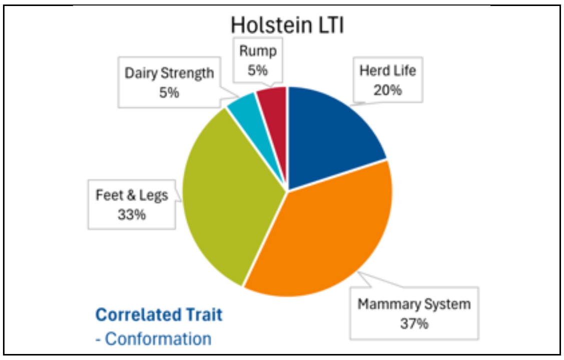


Figure 3. Traits included in the Longevity & Type Index (LTI) of LPI for Holsteins, with their relative weights, and key correlated traits.

Rump and Dairy Strength both have relative weights of 5% for Holsteins to maintain the current genetic level of their underlying traits without targeting further genetic gain per se. Given the fact that overall Conformation is a composite index of the four major scorecard traits, it is monitored only as a correlated trait of this subindex.

Given the relative weights of each trait in the Longevity & Type Index and the relative weights of each subindex in LPI presented in Table 1, the resulting correlations with LPI and the expected selection response (ESR) by trait are presented in Table 3. These results clearly show that applying a relative weight of only 20% on Herd Life still yields the highest LPI correlation and an ESR of 3.4 units of Relative Breeding Value (RBV) for the next five years. Similar to the result discussed for Milk Yield under the Production Index, having no direct weight on Conformation still yields a correlation with LPI of 0.51 and also a relatively strong 5-year ESR of 3.2 EBV units. The key composite traits of Mammary System and Feet & Legs also show moderate correlations with LPI of 0.47 and 0.46, respectively, and associated levels of 5-year ESR.

Table 3: Relative weight (%) of traits included in the Longevity & Type Index (LTI) of LPI for Holsteins, their resulting correlation with LPI, and the expected selection response (ESR) by trait over the next 5 years resulting from selection for LPI.

	%	**LPI Corr**	**ESR**
Herd Life	20	**0.64**	**3.4**
Conformation		**0.51**	**3.2**
Mammary System	37	**0.47**	**2.9**
Feet & Legs	33	**0.46**	**2.7**
Dairy Strength	5	**0.07**	**0.5**
Rump	5	**0.09**	**0.6**

Health & Welfare Index (HWI)

Compared to the previous Health & Fertility component of the LPI formula in Canada, the new Health & Welfare Index was separated out to facilitate the genetic selection and improvement for multiple traits related to disease resistance and animal welfare. With the goal of reducing the incidence of important dairy cattle diseases, Mastitis Resistance and Metabolic Disease Resistance are directly included in the new Health & Welfare subindex with relative weights of 47% and 27%, respectively (Bjelland et al. 2025), as shown in Figure 4. Hoof Health is currently the only family of traits evaluated in Canada related to animal welfare but has a relative weight of 21% in this LPI subindex. While Somatic Cell Score, Metritis and Retained Placenta are correlated traits not directly included in the Health & Welfare Index, Cystic Ovaries is included with a weight of 5% to achieve a desired selection outcome.

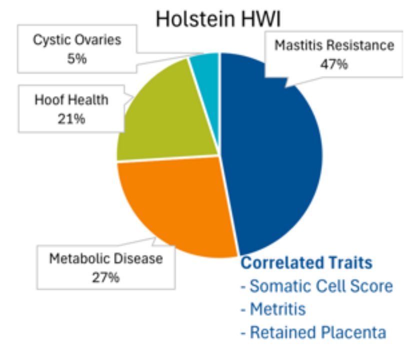


Figure 4. Traits included in the Health & Welfare Index (HWI) of LPI for Holsteins, with their relative weights, and key correlated traits.

Mastitis Resistance is an index that combines Clinical Mastitis with Somatic Cell Score, which is an indicator of sub-clinical mastitis. For this reason, the LPI correlation with these two traits are very similar at 0.44 and 0.46, respectively, and the 5-year ESR exceeds 2 RBV points for both traits (Table 4).

Table 4: Relative weight (%) of traits included in the Health & Welfare Index (HWI) of LPI for Holsteins, their resulting correlation with LPI, and the expected selection response (ESR) by trait over the next 5 years resulting from selection for LPI.

	%	LPI Corr	ESR
Mastitis Resistance	47	**0.44**	**2.1**
Somatic Cell Score		**0.46**	**2.8**
Metabolic Disease Resistance	27	**0.40**	**2.1**
Hoof Health	21	**0.27**	**1.4**
Cystic Ovaries	5	**0.20**	**1.0**
Metritis		**0.37**	**1.9**
Retained Placenta		**0.19**	**1.0**

Compared to these two traits, even though Metabolic Disease Resistance has a lower direct weight of 27% in this subindex, it has a similar LPI correlation of 0.40 and ESR of 2.1 RBV points over the next five years. With a relative weight of 21%, Hoof Health has a relatively low LPI correlation of 0.27, which translates to an ESR of 1.4 RBV points after 5 years of LPI selection.

In Canada, Lactanet provides genetic evaluations for three fertility disorders. For two of them, namely Metritis and Retained Placenta, positive correlations with LPI resulted even without direct emphasis in the Health & Welfare Index (Table 4). This stems from the positive correlations that these traits have with both Herd Life and Daughter Calving Ability, which are directly included in the modernized LPI formula. For Cystic Ovaries, however, which has a relatively low correlation of 0.34 and 0.19 with the other two fertility disorders, respectively, direct inclusion with a 5% relative weight was decided to obtain the desired 5-year ESR of 1.0 RBV points.

Reproduction Index (RI)

The Reproduction Index focuses on improvement of traits related to female fertility, calving ease and calf survival (i.e.: reverse expression of stillbirth rate). Given the major importance of female fertility, the Daughter Fertility index has a relative weight of 90% and 10% is allocated to Daughter Calving Ability (Figure 5). Calving Ability, which is an index that includes service sire traits for calving ease and calf survival, is monitored as a correlated trait to this LPI subindex.

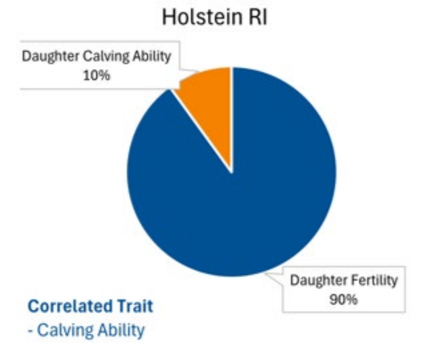


Figure 5. Traits included in the Reproduction Index (RI) of LPI for Holsteins, with their relative weights, and key correlated traits.

As shown in Table 5, even though Daughter Fertility has the highest emphasis in the Reproduction Index, Daughter Calving Ability has the highest correlation with the resulting LPI at 0.58, compared to 0.32 for Daughter Fertility. These correlations translate to 5-year ESR values of 2.8 and 1.6 RBV points, respectively. Without direct inclusion of Calving Ability in this subindex, the LPI correlation slightly exceeds that of Daughter Fertility at 0.34 and the associated ESR is 1.9 RBV points after 5 years of selection for LPI.

Table 5: Relative weight (%) of traits included in the Reproduction Index (RI) of LPI for Holsteins, their resulting correlation with LPI, and the expected selection response (ESR) by trait over the next 5 years resulting from selection for LPI.

	%	LPI Corr	ESR
Daughter Fertility	90	0.32	1.6
Daughter Calving Ability	10	0.58	2.8
Calving Ability	0	0.34	1.9

Milkability Index (MI)

With the growing adoption of robotic milking systems, the objective of creating a new Milkability Index for inclusion in the LPI was to allow dairy farmers to specifically select for a group of traits related to milking ability and efficiency. As shown in Figure 6, Milking Speed and Temperament are directly included in the subindex, with relative weights of 25% and 18%, respectively.

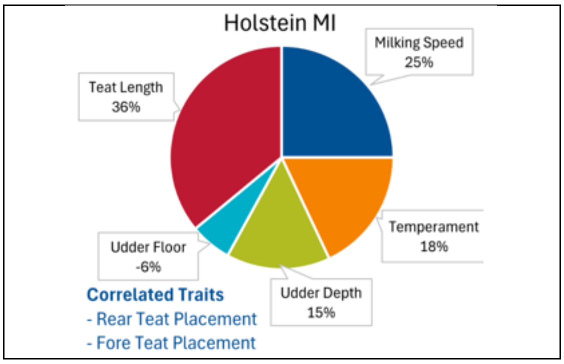


Figure 6. Traits included in the Milkability Index (MI) of LPI for Holsteins, with their relative weights, and key correlated traits.

Multiple descriptive type traits related to the udder and teats were also considered for direct inclusion. The result of various analyses led to the inclusion of Teat Length at 36%, Udder Depth at 15% and Udder Floor at -6%, with the negative value encouraging selection away from udders with a reverse tilt. Rear and Fore Teat Placement are considered as correlated traits.

The relative weights used for directly including Milking Speed and Temperament in the Milkability Index do not, however, lead to strong correlations with LPI (i.e.: 0.03 and 0.10, respectively), and therefore high levels of ESR are not expected, as shown in Table 6.

Table 6: Relative weight (%) of traits included in the Milkability Index (MI) of LPI for Holsteins, their resulting correlation with LPI, and the expected selection response (ESR) by trait over the next 5 years resulting from selection for LPI.

	%	LPI Corr	ESR	
Milking Speed	25	0.03	0.1	
Temperament	18	0.10	0.5	
Udder Depth	15	0.31	2.0	Shallow
Udder Floor	-6	-0.01	-0.1	Tilt
Teat Length	36	-0.19	-1.2	Short
Rear Teat Placement		0.03	0.2	Close
Fore Teat Placement		0.15	0.9	Close

All five of the descriptive type traits considered for inclusion in this subindex are evaluated on an intermediate optimum scale whereby bull EBVs with higher numerical values (i.e.: either above or below zero) reflect sires that will move the breed toward one extreme or the other, with both being undesired. This expression scale means that correlations with these traits need to be carefully interpreted. The 15% emphasis on Udder Depth in the Milkability subindex results in a correlation with LPI of 0.31 based on the 5% relative weight of this subindex in LPI as shown in Table 1. Based on how this trait is measured by the classifiers and designated a linear score from 1 to 9, positive EBV correlations would lead toward more shallow udders instead of deeper. The 5-year ESR for Udder Depth of 2.0 EBV units in the direction of shallow udders is the desired target to reduce problems in robotic and parlour milking systems. For Udder Floor, a negative relative weight of -6% was required to achieve a near-zero LPI correlation and ESR so that selection for LPI would not increase the frequency of reverse tilt udders, which are especially problematic with robotic milkers.

Teat Length was a trait of particular interest since breeders and the industry have taken steps to mitigate the past trend towards shorter teats. For this reason, this trait receives the highest relative weight in the Milkability Index at 36%. Even with this emphasis, however, the resulting correlation between Teat Length and LPI is -0.19, which yields a 5-year ESR of 1.2 EBV units towards shorter teats. Without the 36% emphasis on this trait in this subindex, this suboptimal selection direction would be even stronger. During the industry consultation process, various relative weights for Teat Length were tested but an ESR toward shorter teats was a consistent result. This is caused by the underlying correlation matrix between traits since Teat Length has a moderate negative correlation (i.e.: toward shorter teats) ranging from -0.20 to -0.28 with other traits directly included in the LPI, including Fat Yield, Herd Life, Udder Depth and Daughter Calving Ability. Without any direct inclusion of Rear and Fore Teat Placement in the Milkability Index, the correlation with LPI is either neutral or slightly favourable, at 0.03 and 0.15, respectively, to avoid selection towards teats that become wider apart (Table 6).

Environmental Impact Index (EI)

Since 2021, Lactanet introduced genetic evaluations for three traits directly targeting the reduction of greenhouse gas emissions produced by animals on dairy farms. These include Feed Efficiency and Body Maintenance Requirements, which reflect the volume of feed consumed, and Methane Efficiency that reflects methane yield independent of production levels. As shown in Figure 7, all three of these traits are directly included in the Environmental Impact Index with relative weights of 25%, 38% and 37%, respectively, based on analysis reported by Richardson et al. (2025).

The trait correlations with LPI and ESR based on five years of LPI selection are presented in Table 7. For Methane Efficiency and Feed Efficiency, the LPI correlations are relatively low but in the desired direction at 0.19 and 0.09, respectively. Even with only 5% weight of this subindex in the current LPI, some favourable response is expected with 5-year ESR values of 0.9 and 0.5 RBV points.

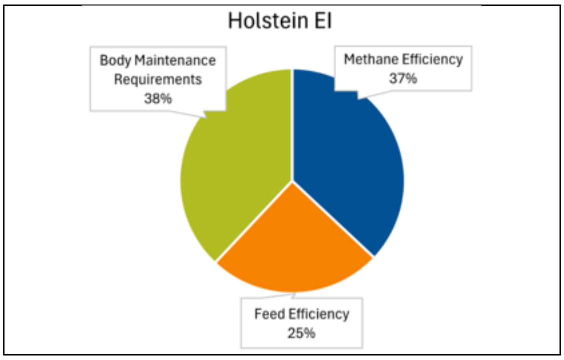


Figure 7. Traits included in the Environmental Impact Index (EI) of LPI for Holsteins, with their relative weights, and key correlated traits.

Table 7: Relative weight (%) of traits included in the Environmental Impact Index (EI) of LPI for Holsteins, their resulting correlation with LPI, and the expected selection response (ESR) by trait over the next 5 years resulting from selection for LPI.

	%	**LPI Corr**	**ESR**
Methane Efficiency	37	**0.19**	**0.9**
Feed Efficiency	25	**0.09**	**0.5**
Body Maintenance Requirements	38	**-0.16**	**-0.8**

The same result is not found for Body Maintenance Requirements, which has an LPI correlation of -0.16 and an ESR of -0.8 after five years of LPI selection (Table 7). Fleming et al. (2023) developed the single step evaluation system for this trait using metabolic body weight as the phenotypic measure. Resulting sire RBV are expressed such that higher values result in the selection of more moderately sized daughters. The genetic trend for this trait has been negative so inclusion in the Environmental Impact subindex of LPI is an important step to at least reducing the rate of the observed genetic trend. As financial incentives are introduced in the future to encourage dairy farmers to reduce the carbon footprint of their herd, it is expected that this subindex will have higher emphasis in the LPI formula.

Expression of LPI Subindexes

Lactanet currently calculates genetic evaluations for over 100 individual traits and indexes. On its LactanetGen.ca website, which provides genetic information and associated tools, over 50 traits are displayed on each animal’s Genetic Evaluation Summary page.

In general, while some breeders have a keen interest in studying the detailed genetic profile of sires and the females in their herd, most dairy farmers are overwhelmed by the number of traits to consider for their selection and mating decisions. To simplify such decisions, each of the six subindexes of Canada's modernized LPI formula are expressed on a standardized scale within each breed and can therefore be used as an overall trait on their own. As presented in Figure 8, each subindex has an average of 500 and a standard deviation of 100 points based on the group of progeny proven sires that forms the genetic base within each breed. For Holsteins, this includes proven bulls born in the most recent complete 10-year period (i.e.: for 2025 includes bulls born from 2009 to 2019). The key advantages of this standardized scale include (a) easily identifies elite sires for each subindex (i.e.: 700 or higher), the higher range results in fewer animals tied at the same level, and (c) the higher average results in all animals in the active population of bulls and females have positive subindex values.

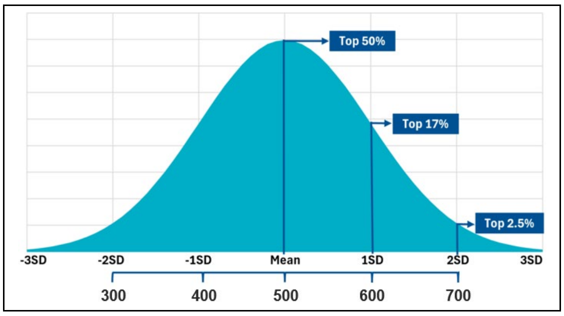


Figure 8. Scale of expression used for each LPI subindex within each breed.

Conclusions

The Lifetime Performance Index (LPI) was Canada's first national genetic selection index introduced in 1991. At that time, only six traits were included to meet the breeding goal of balancing production and type with relative weights of 40:20. Over the past 30+ years, many new traits were introduced, and breeding objectives were broadened. Most recently, the launch of genetic evaluations associated with environmental sustainability served as strong motivation to modernize the LPI formula for all seven dairy breeds evaluated in Canada.

The new modernized LPI maintains focus on production traits with its Production Index, which is followed by the Longevity & Type Index in terms of relative weight. The increased number of evaluations targeting selection for enhanced disease resistance led to the creation of a new Health & Welfare Index. The Reproduction Index was broadened to include calving performance traits in addition to female fertility. With the increase adoption of robotic milking systems, a new Milkability Index was created, which allows dairy farmers to specifically select for key traits in this area. For the Holstein breed, a new Environmental Impact Index combines the three traits currently evaluated and was introduced in the LPI formula to increase awareness and initiate genetic selection for this novel family of traits. Within each breed the subindexes are expressed on a standardized scale to facilitate producer understanding and they are published alongside LPI to give increased visibility.

An important shift with the modernized LPI is the focus towards the communication of expected selection response (ESR) achieved by selection for LPI, rather than concentrating on the list of traits included and their relative weight. This approach is more appropriate for describing the rate of genetic change that can be expected by index selection and accounts for the underlying correlations among the traits and indexes.

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Quantifying the use of and the genetic progress from advanced mating strategies in US dairy herds

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Abstract

The use of mating technologies, including genomic testing and sexed semen, has recently increased in the breeding programs of commercial dairy herds, along with the use of beef semen. We aimed to quantify the utilization of advanced mating strategies in US dairy herds and the influence of these strategies on genetic merit. Breeding records (n = 35,124,479) that resulted in successful pregnancies of cows and heifers by semen type (conventional dairy, sexed dairy, and beef) and records of genomic testing of female dairy cattle were extracted from the National Cooperator Database for the years 2008 to 2023. Herds were categorized within year by semen type used and use of genomic testing of heifers and the genetic merit of heifers born in 2023 (n = 678,064) was compared by herd mating strategy. Female dairy cattle in the US are genotyped, on average, at 6 months of age. When the net merit of a genotyped heifer increased by one standard deviation, the odds that she remained in the herd through first lactation increased by 13%. Breeding values of net merit ($1,203) and most of the traits investigated were most favorable in heifers born in herds that used all mating strategies investigated (genotyping of heifers, and a combination of beef, sexed, and conventional semen). Calves born in herds that used a combination of sexed and conventional semen had the least net merit ($532) and generally had the least favorable breeding values across production, fertility, and longevity traits. Results confirm that the incorporation of advanced mating strategies has increased rapidly in US dairy herds. Heifers were more likely to enter the milking herd as their genomic merit increased and herds that incorporated all strategies investigated had the greatest genetic progress.

Key words: Genomic testing, sexed semen, beef on dairy

Introduction

Dairy cattle breeders have used artificial insemination for many decades; now newer tools, like genomic testing, allow for more precise breeding strategies. Following the inception of genomic selection, rapid genetic progress has occurred in the US dairy cattle population (Garcia-Ruiz et al., 2016; Guinan et al., 2023). Recent reports support that there has been a rapid increase in genotypes of dairy females (CDCB, 2025) and in dairy cattle being mated to sexed and beef semen (Lauber et al., 2023).

Multiple research groups have simulated the economic and genetic benefits of incorporating female genomic testing, sexed semen, beef semen, and combinations thereof into mating programs. For example, models suggest that using genomic information to select replacement heifers can reduce genetic lag through increased selection accuracy and selection intensity (Weigel et al., 2012; Calus et al., 2015). Likewise, selection intensity can be increased by mating genetically superior females to sexed semen, ensuring replacement heifers are born to the best cows (Weigel, 2004; De Vries et al., 2008). Combining sexed semen with genomic testing of heifers increased the rate of genetic progress because more heifers in a herd increased room for selection (Calus et al., 2015).

Because of the added value beef x dairy calves have over dairy bull calves, economic models suggest that profitability can be maximized in dairy mating programs when sexed semen and beef semen are selectively used, though not in herds with poor reproductive performance (Pahmeyer and Britz, 2020; Cabrera, 2022). Additionally, as proportions of sexed and beef semen increased, genetic lag was reduced (Hjortø et al., 2015; Clasen et al., 2021). Using genomic testing to inform selective use of sexed and beef semen further reduced genetic lag, though economic gains were similar in models that did not use genomic testing (Hjortø et al., 2015; Clasen et al., 2021).

The combined use of genomic testing, sexed semen, and beef semen in US dairy herds and their influence on genetic progress has yet to be quantified. We aimed to characterize the utilization of advanced breeding strategies in US dairy herds by quantifying genotyping of replacement heifers, and the use of conventional, sexed, and beef semen, and combinations thereof. Further, we sought to test the hypothesis that herds that use combinations of advanced breeding tools produce replacement dairy calves with greater genetic merit than those using exclusively conventional semen.

Materials and Methods

The data used in this study was accessed from the National Cooperator Database, managed by the Council on Dairy Cattle Breeding (CDCB). The phenotypes, genotypes, and pedigree used in the US national dairy cattle genetic evaluation and this study included phenotypes of reproductive events (Format 5), herd test-date records, and genotypes of females born in the US.

Predicted breeding values (PBV) on the lifetime net merit (NM$ index and PBV of traits evaluated in all dairy breeds from the August 2024 official national genetic evaluation were extracted. All dairy breeds were included in this study, including crossbred animals. Heifers and cows were classified by the breed base PBV are reported on (Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, or Milking Shorthorn), when relevant.

Heifer genotypes

Between 2008 and 2023, a total of 5,683,150 individual female dairy cattle born in the United States were genotyped. The earliest instance of genotyping was retained to determine the initial age a farmer intended to genotype a female calf.

Breeding strategies

Breeding events of cows and heifers that were extracted from Format 5 reproductive records submitted by dairy records processing centers. Events that resulted in full-term pregnancies, verified by a calving event that occurred within breed average gestation length ± 14 days were retained. Data submitted from herds that use less than 80% AI were removed. Miles et al. (2023) recently reported that there are no Format 5 breeding records associated with 97% of calves born via embryo transfer (ET). Due to these data flow and quality issues, we did not attempt to quantify calvings from ET events and animals conceived through ET were removed from the data. Retained AI breeding events that resulted in calvings between the years 2008 and 2023 (n = 35,124,479) were used to quantify changes in semen type use over time.

A subset of the genotyped females (n = 982,536) was examined to determine the proportion of genotyped heifers that remained in the herd they were born in through their first calving. For inclusion, cattle had to be genotyped as heifers ($\leq$24 months of age) to allow for a culling decision to be made prior to their first calving. Likewise, calves were required to be born prior to 2022 to provide adequate opportunity to become cows. To fairly determine that a heifer reached first lactation, only animals born in herds that had at least one DHIA test in 2023 and 2024 were included. Genotyped calves that began their first lactation in the same herd they were born in were denoted as stay = 1, while those that started their first lactation in a different herd or had no associated lactation records were denoted as stay = 0.

Breedings were classified by 4 semen types: conventional dairy-breed semen, sexed (X-sorted) dairy-breed semen, conventional beef-breed semen, and sexed (Y-sorted) beef-breed semen. Breeding strategies were categorized by herd-year and defined to capture both genomic testing of heifers and types of semen used to conceive the calves born within the herd-year. Herds were binned by semen type within year as follows: conventional (CON), where calves born were exclusively conceived with conventional dairy-breed semen; beef and conventional (BC), where calves born were conceived with conventional dairy-breed semen or with beef-breed semen that could be conventional or sex-sorted; sexed and conventional (SC), where calves born were conceived with sex-sorted or conventional dairy-breed semen; beef, sexed, and conventional (BSC), where calves born were conceived with sex-sorted or conventional dairy-breed semen or beef-breed semen. Additionally, SC and BSC herds that utilized genomic testing (GT) were considered those that genotyped any heifers born in the year observed and were binned separately by semen type as GT-SC, and GT-BSC.

To compare the genetic merit of heifers by herd breeding strategy calving events, dairy heifer calves born in 2023 (n = 678,064) were categorized by the breeding strategy of the herd they were born in.

Statistical analyses

The effect of genetic merit on whether a genotyped calf was sold prior to first lactation was evaluated with following binomial generalized linear mixed model:

$$log\left[\frac{p}{(1-p)}\right] = \mu + \beta_1 NM_i + \beta_2 age_j + herd_k + \varepsilon_{ijkl}$$

where p = the probability of $y_{ijkl} = 1$, where y = heifer stayed in herd through first lactation; μ = model intercept; β_1 = regression coefficient of stay on NM$; β_2 = regression coefficient of stay on age genotyped; $herd_k$ = the random effect of the herd the heifer was born in *k* (herd 1 to herd 2,030); and ε_{ijkl} = residual error.

The odds ratio (OR) of a heifer staying in the herd when NM$ increased by 1 SD was generated with a 95% CI.

Merit index PBV and PBV of individual traits of heifer calves born in 2023 were compared by herd mating strategy with the following linear mixed model:

$$y_{ijklm} = \mu + MS_i + lact_j + BB_k + S_l + herd_m(MS_i) + \varepsilon_{ijklm}$$

where y = calf PBV; μ = model intercept; MS_i = mating strategy used by the herd the calf was born in *i* (CON, BC, SC, BSC, GT-SC, GT-BSC); $lact_j$ = dam parity *j* (1, 2, 3, 4, or ≥5); BB_k = the breed base PBV of the heifer calf are reported on *k* (Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, or Milking Shorthorn); S_l = the random effect of season calf was born in two-month intervals (1 to 6); $herd_m$ = the random effect of herd *m* (herd 1 to herd 5613) nested within mating strategy *i*; and ε_{ijklm} = residual error.

Differences in least squares means were Tukey-Kramer adjusted for multiple comparisons. Statistical analyses were performed with SAS (9.4). Data visualization was conducted in R (v. 4.4.1) using the ggplot2 package (Wickham, 2016).

Results & Discussion

Heifer genotypes

In 2008, 68% of female dairy cattle genotyped were 24 months or older; average age at genotyping was 42.4 months (Figure 1). By 2009, just less than 1/3 of females were 2 years of age or older when genotyped. The proportion of heifers (≤ 24 mo. old) genotyped increased each year. In 2023, average age at genotyping was lowest at 5.5 months. Over all 15 years of data, average age at genotyping was 6.3 ± 8.5 months.

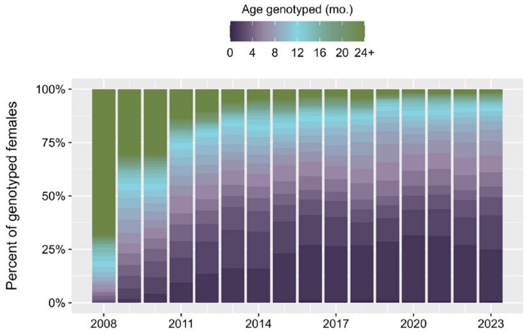

Figure 1. The age US female dairy cattle were genotyped as the percentage of total females genotyped in a year.

Nearly all genotyped females in the US were genotyped as heifers, which aligns with recommendations in the literature to maximize economic return of genomic testing. In simulated data, selecting females for breeding by GEBV resulted in a gain in NM$, following deduction of the cost of genotyping, over selecting females based on parent average (Weigel et al., 2012). However, the increase in genetic merit was reduced when females were genotyped as cows, thus, authors recommended genotyping calves and heifers for the greatest return on investment (Weigel et al., 2012). By genotyping youngstock, farmers can also leverage genomic information to make mating decisions for an animal in future parities, which Hjortø et al. (2015) demonstrated increases the economic returns of genotyping.

The odds that a genotyped heifer calf stayed through first lactation when NM$ increased by 1 SD of the mean ($511) increased by 13.6% (OR = 1.136 [1.131,1.141]). We expected that greater NM$ would increase the likelihood that a heifer stayed in the herd, but the magnitude is smaller than anticipated. This may indicate that management strategies implemented with genomic results vary across herds. In a scenario where a herd utilizing genomic testing has surplus heifers, culling excess heifers with the least genetic merit is a logical selection strategy. However, some herds may choose to market their genetically elite heifers as breeding stock while retaining females with lower genetic merit for use as embryo recipients or for mating with beef semen.

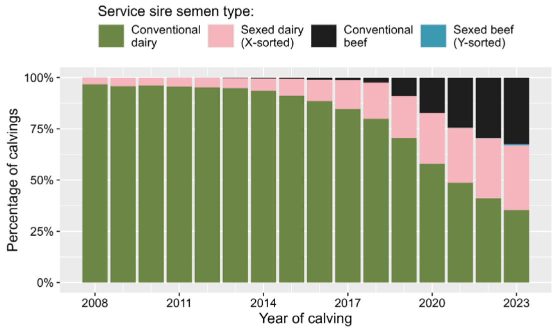

Figure 2. The proportion of annual calvings in US dairy herds by service sire semen type.

Breeding strategies

Over 90% of the calves born from 2008 to 2015 were conceived with conventional semen, with most of the remaining of calves conceived with sexed dairy semen (Figure 2). In 2016, 1% of the calves born were beef-sired and the proportion of calves conceived with sexed semen grew to 10%. By 2019, calvings to beef service sires grew to 9%, and calvings to sexed dairy semen grew to 21%. The proportions of calvings to beef and sexed semen continued to grow through 2023, when the calves born were conceived with nearly equal proportions of beef, sexed, and conventional semen. Additionally, in 2023, about 0.5% of calvings resulted from insemination with sexed (Y-sorted) beef semen to produce terminal beef × dairy steers. Lauber et al. (2023) reported a similar year-over-year increase in proportion of US Holsteins and Jerseys mated to beef and sexed semen from 2019 to 2021.

Calf genetic merit

Calves born in GT-BSC herds had the greatest NM$ while calves born in SC herds had the least (Table 1). The NM$ breeding value of calves born in GT-BSC was $240 greater than calves born in BSC herds. A smaller difference

Table 1. Breeding values of heifers born in 2023 by the mating strategy[1] used by the herd they were born in.

	Herd mating strategy[2]						
	CON	BC	SC	BSC	GT-SC	GT-BSC	SE
n heifers	25,264	26,684	32,902	279,271	17,604	296,335	-
(n herds)	(1,117)	(786)	(891)	(1,810)	(231)	(778)	
PBV							
Net merit, $	678^{d}	857^{c}	532^{e}	963^{b}	678^{d}	1203^{a}	37
Milk, kg	849^{c}	991^{b}	689^{d}	1,019^{b}	714^{d}	1,091^{a}	34
Fat, kg	30.4^{d}	37.1^{c}	25.1^{e}	42.1^{b}	31.4^{d}	51.6^{a}	1.6
Protein, kg	27.7^{d}	32.7^{c}	23.1^{e}	35.2^{b}	25.9^{de}	40.1^{a}	1.1
Somatic cell score	2.90^{b}	2.87^{c}	2.93^{a}	2.86^{c}	2.90^{b}	2.82^{d}	0.01
Productive life, mo.	3.01^{d}	3.80^{c}	2.57^{e}	4.44^{b}	3.53^{c}	6.01^{a}	0.19
Livability, %	-0.13^{c}	0.53^{b}	-1.19^{d}	0.62^{b}	-1.04^{d}	1.26^{a}	0.16
Daughter pregnancy rate, %	-0.97^{bc}	-0.85^{ab}	-1.21^{d}	-0.81^{a}	-1.23^{cd}	-0.71^{a}	0.09
Cow conception rate, %	-0.50^{d}	-0.17^{c}	-0.98^{e}	0.10^{b}	-0.86^{e}	0.60^{a}	0.12
Heifer conception rate, %	1.83^{d}	2.01^{c}	1.78^{d}	2.35^{b}	1.85^{cd}	2.82^{a}	0.09
Early first calving, days	8.34^{d}	9.63^{c}	6.97^{e}	10.19^{b}	6.83^{e}	11.36^{a}	0.28
Body weight composite	0.51^{b}	0.29^{c}	0.86^{a}	0.26^{c}	0.87^{a}	0.00^{d}	0.06
Udder composite	0.90^{d}	0.85^{d}	1.39^{b}	1.03^{c}	1.69^{a}	1.11^{c}	0.06
Feet and leg composite	0.54^{b}	0.42^{c}	0.90^{a}	0.48^{bc}	1.05^{a}	0.42^{c}	0.05

[1] All included model effects, including herd mating strategy, were significant at $P < 0.0001$.

[2] CON = calves born to conventional semen only; BC = calves born to beef and conventional semen; SC = calves born to sexed and conventional semen; BSC = calves born to beef, sexed, and conventional semen; GT-SC = calves born to sexed and conventional semen and some heifers were genotyped; GT-BSC = calves born to beef, sexed, and conventional semen and some heifers were genotyped.

[a,b,c,d,e] Values within row with different superscript are different at $P < 0.05$.

($106) in heifer calf NM$ existed between BSC herds and BC herds, while NM$ of calves born in BC herds was $179 greater than that of calves born in CON and GT-SC herds.

The PBV of production (milk, fat, protein, and somatic cell score), longevity (productive life and livability), and fertility (daughter pregnancy rate, cow conception rate, heifer conception rate, and early first calving) traits of heifer calves generally ranked in the same order of NM$ by herd breeding strategy. Across these trait groups, breeding strategies from most to least favorable genetic merit ranked as follows: GT-BSC, BSC, BC, CON, GT-SC, and SC (Table 1). This is expected because these traits are included in the NM$ index (VanRaden et al., 2021).

An unexpected result was that calves born in CON herds had similar or greater NM$ and PBV of production, fertility, and longevity traits than heifers born in GT-SC and SC herds. The use of sexed semen in the dairy herd is expected to increase the rate of genetic progress by increasing selection intensity on the dams of cows selection pathway (Weigel, 2004; De Vries et al., 2008). Increased genetic progress with sexed semen use is clear for GT-BSC and BSC herds but not in GT-SC and SC herds. We theorize that this is due to the breeding goals of SC and GT-SC herd differing substantially from the herds using other breeding strategies investigated.

Among type trait composites, genetic merit of body weight composite (BWC) ranked by herd mating strategy similarly to that of NM$. Heifers born in GT-BSC herds had the most favorable (least) BWC PBV and those in SC and GT-SC herds had the least favorable (Table 1). Conversely, heifers born in GT-SC herds had the greatest PBV for udder composite and feet and legs composite, followed by calves born in SC herds (Table 1). This may suggest that SC and GT-SC herds are selecting primarily for improvement in type traits, while herds utilizing other breeding strategies select for genetic improvement in many economically relevant traits. In NM$, increased BWC PBV is not economically favorable because heavier cows require additional feed for growth and maintenance (VanRaden et al., 2021). However, breed association classification scores do not penalize animal size and, in elite cattle shows, tall, large-framed cows are often favored. Thus, in herds prioritizing selection for conformation, greater BWC may not be considered unfavorable.

Conclusions

Genomic testing of heifer calves and the incorporation of sexed and beef semen in mating programs have increased rapidly in US dairy herds. On average, female dairy cattle in the US are genotyped at 6 months of age. The odds that a genotyped heifer remained in the herd through first lactation increased slightly (by 13%) when her NM$ PBV increased by $511, suggesting that knowledge of genetic merit from genotypes may have informed replacement selection. Heifers born in herds that used all mating strategies investigated (genotyping of heifers, and a combination of beef, sexed, and conventional semen) had the greatest genetic merit when measured on the four merit selection indexes and across most PBV investigated. Calves born in SC and GT-SC had the least genetic merit across production, fertility, and longevity traits but had the greatest merit for udder and feet and legs conformation, perhaps due to different breeding objectives. Dairy herds that combine advanced mating strategies generally produce genetically superior replacement heifers.

Acknowledgments

A longer, peer reviewed version of this research report has since been published: 10.3168/jds.2025-26600. This work was supported by USDA Agricultural Research Service (ARS) CRIS project 8042-31000-113-000-D (Beltsville, MD) and in part by an appointment to the ARS Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the US Department of Energy (DOE) and the USDA. ORISE is managed by ORAU under DOE contract number DE-SC0014664. Data were available to the authors from CDCB (Bowie, MD) under USDA Agricultural Research Service Material Transfer Research Agreement #58-8042-8-007. While CDCB offers data stewardship, sole ownership and rights pertaining thereto remain with the producer and we thank US dairy producers for sharing their data for research use. All opinions expressed in this paper are the author's and do not necessarily reflect the policies and views of USDA, DOE, or ORAU/ORISE. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA (Washington, D.C.). The authors have not stated any conflicts of interest.

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Renewed Genetic Evaluation of Heat Tolerance in Italian Holsteins

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Abstract

Heat stress is a significant and growing challenge for the Italian dairy industry, adversely affecting milk production, fertility, and animal welfare. This study presents a renewed genetic evaluation for heat tolerance in Italian Holstein cattle, expanding upon a previous 2021 index that focused solely on milk yield. The primary objective was to develop a more comprehensive selection tool by incorporating five production traits: milk yield (kg), fat yield (kg), protein yield (kg), fat percentage, and protein percentage. Test-day production records were augmented with meteorological data from 137 weather stations across Italy to calculate a 7-day average Temperature-Humidity Index (THI) for each record. A repeatability mixed model was first employed to identify the specific THI thresholds at which each of the five traits begins to decline. Subsequently, a random regression mixed model was implemented to estimate genetic parameters and calculate Estimated Breeding Values (EBVs) for both general production merit and specific heat tolerance. THI thresholds were identified for all traits, with milk yield declining above a THI of 70. Heritabilities for heat tolerance traits were found to be low to moderate, ranging from 0.12 for fat yield to 0.37 for protein percentage, indicating sufficient genetic variation for selection. An aggregate index, the Heat Tolerance Index (IHT), was developed by assigning economic weights to each trait Heat Tolerance EBV. Validation results demonstrated the index's efficacy: daughters of high-IHT bulls (+1 SD) lost 0.91 kg/d less milk during summer compared to daughters of low-IHT bulls (-1 SD). This renewed evaluation provides a robust tool to select for more resilient animals, offering a tangible strategy to mitigate economic losses and improve animal welfare in a warming climate.

Key words: heat tolerance, genetic evaluation, Holstein, THI, milk production, regression

Introduction

Heat stress poses a substantial threat to the dairy industry worldwide, leading to significant reductions in milk yield, impaired reproductive performance, and compromised animal welfare. As global temperatures continue to rise, these challenges are becoming increasingly prevalent, particularly in Mediterranean climates like in Italy. The economic consequences of heat stress are severe. In Italy, with a population of approximately one million Holstein cows, summer-related production losses are estimated to be around 1.5 kg of milk per cow per day over a 180-day period, culminating in an annual loss of approximately 270,000 tons of milk.

While management strategies such as cooling systems can alleviate some effects, they represent a recurring cost. Genetic selection offers a cumulative and permanent solution by breeding cows that are naturally more resilient to heat stress. In 2021, a heat tolerance index based solely on milk yield was introduced for Italian Holsteins (Finocchiaro et al., 2022). To enhance the selection process, a more robust and comprehensive evaluation was desired.

The objectives of this study were therefore to: 1) expand the genetic evaluation for heat tolerance to include five key production traits: milk (kg/d), fat (kg/d and %), and protein (kg/d and %); 2) determine the specific Temperature-Humidity Index (THI) thresholds at which these traits begin to decline; 3) estimate the heritability of heat tolerance for each trait; and 4) develop and validate a new aggregate selection index (IHT) to improve heat resilience in Italian Holstein cattle.

Materials and Methods

Data Source and Preparation

Test-day production records for milk, fat, and protein yields from first, second, and third lactation Italian Holstein cows were obtained from the national database of the Italian Holstein Friesian and Jersey Breeders Association (ANAFIBJ).

Meteorological data, including daily maximum temperature and relative humidity, were collected from 137 weather stations across Italy for the period starting in 1994. Herds were assigned geographic coordinates based on their municipality, and each herd was linked to an average of 2.3 nearby weather stations, with an average distance of 13.5 km. For each test-day record, a corresponding Temperature-Humidity Index (THI) was calculated using the formula from Kelly and Bond (1971). To account for the cumulative effects of heat, a 7-day average THI preceding the test day was used for all analyses.

Statistical Analyses

THI Threshold Identification

To identify the critical THI value above which production traits decline, a repeatability model was fitted using ASReml software. The model was:

$$\boldsymbol{Y = HYS + YC + DIM * age * parity + THI + a + pe + e}$$

where **Y** is the phenotype for a given trait; **HYS** is the fixed effect of herd-year-season of test day; **YC** is the fixed effect of year of calving; **DIM*age*parity** is the fixed effect for the interaction of days in milk, age at calving, and parity (1, 2, 3); **THI** is the linear regression on the THI value; **a** is the random additive genetic animal effect; **pe** is the random permanent environmental effect; and **e** is the random residual.

Genetic Parameter Estimation

A random regression model was used to estimate genetic parameters and breeding values for heat tolerance using MiX99 software. The model equation was:

$$\boldsymbol{Y = HYS + YC + DIM * age * parity + a + \alpha(f(THI)) + pe + \beta(f(THI)) + e}$$

The fixed effects are as described above. The function **f(THI)** models the heat stress effect as a linear slope only when the THI exceeds the predetermined threshold for that trait:

$$f(THI) = \begin{cases} 0, \mathrm{THI} \le \mathrm{THI}_{\mathrm{threshold}} \\ \mathrm{THI} - \mathrm{THI}_{\mathrm{threshold}}, \mathrm{THI} > \mathrm{THI}_{\mathrm{threshold}} \end{cases}$$

The random effects include the general additive genetic merit **a**, the specific genetic effect for heat tolerance **α(f(THI))**, the permanent environmental effect **pe**, and the permanent environmental effect related to heat tolerance **β(f(THI))**.

Heat Tolerance Index (IHT)

Estimated Breeding Values (EBVs) for heat tolerance (**α**) were calculated for all five production traits. These individual EBVs were then combined into an aggregate Heat

Tolerance Index (IHT). The weights assigned to each trait were: 45% for protein kg, 25% for milk kg, 15% for fat kg, 10% for protein %, and 5% for fat %. The final IHT EBVs were standardized to a mean of 100 and a standard deviation of 5.

Results & Discussion

THI Thresholds and Genetic Parameters

The analysis identified distinct THI thresholds at which different production traits begin to decline (Table 1). Milk yield was the most resilient, with a decline observed only when the THI exceeded 70. Protein and fat components, both in kilograms and percentage, were affected at lower THI values, with thresholds ranging from 52 to 59. This suggests that metabolic changes affecting milk composition occur before a substantial drop in milk volume.

Table 1: THI thresholds for decline in milk production traits.

Milk production trait	Threshold level
Milk (kg/d)	70
Protein (kg/d)	59
Fat (kg/d)	52
Protein %	55
Fat %	52

The estimated heritabilities (h^2) for heat tolerance and genetic correlations are presented in Table 2. Heritabilities ranged from 0.12 for fat kg to 0.37 for protein %, indicating that there is sufficient genetic variation to achieve progress through selection. The genetic correlations between general production merit and heat tolerance were consistently moderate and negative (from -0.42 to -0.51). This antagonism implies that selection solely for high production may lead to a slight decline in heat tolerance, reinforcing the need for a balanced, multi-trait breeding goal.

Table 2: Heritabilities (h^2) for heat tolerance and genetic correlations (r_g) with general production merit.

Milk production trait	Genetic Correlation (r_g)	Heritability (h^2)
Milk (kg/d)	-0.51	0.16
Protein (kg/d)	-0.48	0.13
Fat (kg/d)	-0.42	0.12
Protein %	-0.43	0.37
Fat %	-0.50	0.26

Validation of the Heat Tolerance Index (IHT)

To validate the IHT, the performance of daughters from bulls with high heat tolerance (IHT ≥ +1 SD) was compared to that of daughters from bulls with low heat tolerance (IHT ≤ -1 SD). The comparison focused on the difference in milk yield between summer and winter test days (Table 3) (Flamenbaum, 2016).

Daughters of low-tolerance bulls experienced a substantial drop in production of -1.24 kg/d during the summer. In contrast, daughters of high-tolerance bulls showed a much smaller decline of only -0.33 kg/d. This resulted in a net difference of +0.91 kg/d in favor of the high-IHT group, providing strong evidence that the IHT effectively identifies sires whose progeny are more resilient to heat stress. This difference represents a significant economic advantage and a notable improvement in animal welfare.

Table 3: Comparison of summer vs. winter milk yield loss in daughters of high and low IHT bulls.

Group	Winter milk (kg/d)	Summer milk (kg/d)	Difference (kg/d)
High HT (≥ +1 SD)	30.38	30.05	-0.33
Low HT (≤ -1 SD)	31.14	29.90	-1.24

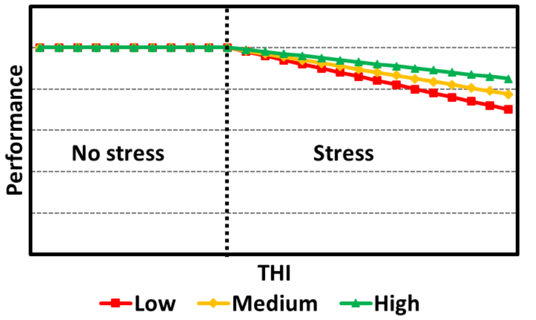

Figure 1. Example of heat stress for low, medium, high tolerance cows

Conclusions

This study successfully developed and implemented a renewed, multi-trait genomic evaluation for heat tolerance in Italian Holstein cattle. By analyzing milk, fat, and protein traits, the evaluation provides a comprehensive assessment of an animal's ability to maintain productivity under heat stress conditions. The resulting Heat Tolerance Index (IHT) has been validated as an effective tool for identifying genetically superior animals. The daughters of high-IHT bulls demonstrate significantly lower milk production losses during hot summer months. The adoption of the IHT in the national breeding program offers a powerful, sustainable, and cumulative strategy to enhance the resilience of the Italian Holstein population, thereby reducing economic losses and improving animal welfare in the face of ongoing climate change.

Acknowledgments

This study was supported by “Latteco2 project, sottomisura 10.2 of the PSRN-Biodiversity 2020–2023” (MIPAAF. D.M. no. 465907 del 24/09/2021, project unique code J12C21004080005).

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Nordic Genetic Evaluation of Feed Efficiency – Updated Model for Saved Feed in Nordic Dairy Cattle Breeds.

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Abstract

Since 2019, the Nordic Cattle Genetic Evaluation (**NAV**) has published breeding values for Saved Feed for Holstein (**HOL**), Jersey (**JER**), and Red Dairy Cattle (**RDC**). This trait is integrated into the Nordic Total Merit (**NTM**) index. Previously, the genetic evaluation of energy use for maintenance was based on body weight and indicator traits such as stature, body depth, and body width. Metabolic efficiency was genetically evaluated by using a two-step model, where the first step is a pre-correction of phenotypes that introduced challenges. This study developed a one-step genomic model for Saved Feed to both address these challenges and improve the use of research and Cattle Feed InTake (**CFIT**) data. The dataset comprised 741,491 weekly records from 4,541 JER, 5,377 RDC, and 8,030 HOL cows. SNP genotypes from NAV were used to create breed-specific single-step genomic evaluations. We fitted two-trait random regression models for each of the traits of dry matter intake (**DMI**), energy-corrected milk (**ECM**), and body weight (**BW**) where we treated the first and later lactations as separate traits. The breeding values for BW change (**ΔBW**) were derived from the BW model. Across lactation (2–44 weeks in milk), the heritabilities ranged from moderate to moderately high for DMI and ECM (0.22–0.50 & 0.47- 0.52) and were moderately high for BW (0.46–0.61). The genetic correlations were strong across parities (≥0.82). These genetic parameters estimated with pedigree based BLUP, were used in three single-step GBLUP models, where lactation-wise breeding values for Saved Feed were calculated within each breed as:

$$\mathrm{GEBV}_{\mathrm{Saved\ Feed}} = 0.40 \times \mathrm{GEBV}_{\mathrm{ECM}} + 4.0 \times \mathrm{GEBV}_{\Delta \mathrm{BW}} - \mathrm{GEBV}_{\mathrm{DMI}}$$

One index unit of Saved Feed corresponds to 18.3 kg of dry matter saved per 305-day lactation, or 183 kg for 10 index units. Among for candidate bulls born in year 2022, the breeding values for Saved Feed had moderate index correlations with the NTM (0.20–0.30), weak to moderate index correlations with yield trait (0.07–0.26), and low index correlations with female fertility, udder health, and general health (–0.14 to 0.12). For HOL and RDC, the index correlations between Saved Feed and frame size were moderately negative (–0.19 to –0.29), while for JER, this correlation was close to zero. In conclusion, NTM has been updated with the new NAV one-step Saved Feed index which should promote genetic progress for feed efficiency in the Nordic Dairy Cattle breeds.

Key words: Saved Feed, Feed Efficiency, Genomic Prediction, Holstein, Jersey, Red Dairy Cattle.

Introduction

Feed is the largest operating expense on dairy farms (Stephansen et al., 2021a) and a major contributor to dairy farms´ greenhouse gas emissions (Kristensen et al., 2015). Consequently, genetic evaluation centers worldwide have developed breeding values for feed efficiency. The Nordic Cattle Genetic Evaluation (**NAV**) has introduced breeding values for Saved Feed by first including breeding values for energy use related to maintenance in 2019 (Lidauer et al., 2019) and then including breeding values for metabolic efficiency in 2021 (Stephansen et al., 2021b). The Saved Feed index has been integrated into the Nordic Total Merit (**NTM**) index since 2020 to support selection for more feed-efficient cows in Holstein (**HOL**), Jersey (**JER**), and Red Dairy Cattle (**RDC**) populations.

Historically, the breeding values for energy use related to maintenance was calculated using body weight data collected from milking robots, heart girth collected with tape measurements, and indicator traits such as stature, body depth, and chest width. The breeding values for metabolic efficiency were calculated as residual feed intakes (**RFI**) using the data from both commercial farms from Cattle Feed InTake (**CFIT**) (Lassen et al., 2023) and research farms from AU-Foulum, Denmark and Luke, Finland. The breeding values for RFI were calculated using a two-step approach, where the first step was a pre-correction using a linear model, from which the residual was used in a single-step GBLUP. However, this two-step approach presented challenges with unrealistic regression coefficient of feed intake on milk energy, difficulty with handling of missing data, and poor correction of fixed effect when using multiple traits in regression models due to different means and variances in different levels of fixed effects. Such issues have previously been documented for this type of RFI model (Tempelman & Lu, 2020; Stephansen et al., 2024).

To address these limitations, we propose a one-step Saved Feed model that was inspired by the works of Khanal et al. (2022) and Abdalla et al. (2024). The objective of this study was to develop and implement a one-step Saved Feed evaluation model that enhanced the accuracy and robustness of breeding values for feed efficiency in the NAV evaluation.

Materials and Methods

Animal Care and Ethics Committee approval was not required for this study as data was collected using standard dairy herd management practices. Furthermore, no treatment or handling of animals were administered during the data collection for this study.

Phenotypic data

The analysis included phenotypic data on all breeds from 24 Danish CFIT farms that were obtained between January, 2019 and December, 2024, phenotypic data on HOL that were obtained from the Danish Cattle Research Center (**DCRC**) between January, 2003 and March, 2022 (Li et al., 2017; Stephansen et al., 2023), and phenotypic data on RDC from Natural Resources Institute Finland farms between September, 1998 and January, 2022 (**Luke**; Mehtiö et al., 2018). The phenotypic data was comprised of weekly averages of dry matter intake (**DMI**), weekly averages of body weight (**BW**), and monthly test-day records for energy-corrected milk (**ECM**). In total, the dataset included 741,491 DMI records from 4,541 JER cows, 5,377 RDC cows, and 8,030 HOL cows (Table 1).

Table 1: Numbers of records, cows, and cows with genotypes in the phenotypic data.

Breed	n records		n cows (genotyped)
	DMI	ECM	
HOL	361,412	202,360	8,030 (5,104)
RDC	233,867	108,255	5,377 (3,828)
JER	146,215	84,486	4,541 (3,040)
Total	741,491	395,101	17,948 (11,972)

HOL=Holstein, RDC=Red Dairy Cattle, JER=Jersey, DMI=Dry Matter Intake, ECM=Energy Corrected Milk

Pedigrees and genotypes

Both the initial breed-specific pedigrees and the genotypic data were provided by NAV using its standard operations from routine evaluations. The initial pedigree was pruned such that it only contained cows with either phenotypic or genotyped individuals and their ancestors within three generations. Afterwards, we added genetic groups for individuals with unknown parents. The genetic groups were defined based on the sex, breed, country of origin, and birth year class of the individual. The final pedigrees included 1,120,681 HOL, 488,855 RDC, and 256,551 JER animals. The genotypic data contained information on approximately 45,000 SNPs. The genotyping rates for animals in the pedigree were 58% for HOL, 66% for JER and 58% for RDC.

Statistical models

DMI, ECM, and BW were analyzed using breed-specific two-trait random regression models where primiparous and multiparous lactations were treated different traits:

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

where $\mathbf{y}$ is a vector of phenotypes for DMI, ECM, or BW across the two parity groups (primiparous or multiparous) and weeks in lactation (week 0-45); $\mathbf{b}$ is a vector of fixed effects for age at first calving (only primiparous), parity (only multiparous), calving herd × calving year × calving season, the regression on lactation curve (5th order Legendre polynomial terms nested within herd); $\mathbf{a}$ is a vector of additive genetic random regression coefficients (Legendre polynomials: intercept, linear); $\mathbf{pe}$ is a vector of permanent environmental random regression coefficients (Legendre polynomials: intercept, linear, quadratic); $\mathbf{p}$ is a vector of random effects for animal × parity (only multiparous); $\mathbf{e}$ is a vector of residuals nested within trait; and $\mathbf{X}$, $\mathbf{Z}$, $\mathbf{W}$, and $\mathbf{M}$ are design matrices. The parameters of the model were estimated using AI-REML in DMU (Madsen and Jensen, 2013) with a pedigree-based relationship matrix.

The estimated (co)variance components were used to predict lactation-wise genomic breeding values for DMI, ECM and BW using the ssGTaBLUP model in the MiX99 software (Mäntysaari et al., 2017). For RDC, we predicted genomic breeding values with (co)variance components from HOL. The genomic breeding values for BW change (**ΔBW**) were derived from the BW model as the difference between GEBVs at day 30 in milk and day 280 in milk.

The new Saved Feed calculation

We used the GEBVs of the traits to calculate breeding values for Saved Feed within each combination of parity group and breed:

$$GEBV_{\text{Saved Feed}} = 0.40 \times GEBV_{\text{ECM}} + 4.0 \times GEBV_{\Delta\text{BW}} - GEBV_{\text{DMI}}$$

Instead of estimating the regression coefficients for ECM and ΔBW in the equation above from genetic parameters, we obtained the regression coefficient for ECM from Abdalla et al. (2024), while the regression coefficient for ΔBW was based on Lidauer et al. (2023). Parity-specific $GEBV_{\text{Saved Feed}}$ values were weighted with 1/3 emphasis on primiparous lactation and 2/3 emphasis on multiparous lactations.

The breeding values for Saved Feed were standardized to have a mean of 100 and a standard deviation (**SD**) of 10 for base animals, defined as three to five years old (rolling base) cows with phenotypes. One index unit represents 18.3 kg of dry matter feed saved in the first 305 days of lactation.

Results & Discussion

Covariance parameters

Lactation-wise (2 to 44 weeks in milk) heritabilities were moderate for DMI (HOL_{primi}:0.43; HOL_{multi}:0.49; JER_{primi}:0.22; JER_{multi}:0.50), moderate for ECM (HOL_{primi}:0.50; HOL_{multi}:0.48; JER_{primi}:0.47; JER_{multi}:0.52), and moderately high for BW (HOL_{primi}:0.52;

$\text{HOL}_{\text{multi}}$:0.58; $\text{JER}_{\text{primi}}$:0.46; $\text{JER}_{\text{multi}}$:0.61). The heritabilities were in accordance with those reported using a CFIT dataset (Stephansen et al., 2025) and research data for HOL in US (Khanal et al., 2022). The genetic correlations between parity groups were high for DMI (HOL: 0.90; JER: 0.82), high for ECM (HOL: 0.89; JER: 0.94), and high for BW (HOL: 0.95; JER: 0.90). This is also in accordance with previous studies (de Jong et al., 2019; Jamrozik et al., 2022; Stephansen et al., 2025).

Index correlations and genetic trends

The new index for Saved Feed had correlations with the previous index of 0.30, 0.50, and 0.25 for HOL, RDC and JER respectively. The levels of these correlations were expected because the model changed to a one-step Saved Feed approach instead of the previous two-step RFI model with challenges observed in the precorrection step, and because we thereby omitted BW phenotypes from cows without DMI and ECM information. The NAV Saved Feed index has been weighted fully into NTM since February 2025 using the economic weights proposed by Sørensen et al., (2018). This has resulted in moderate index correlations between Saved Feed and NTM at 0.30, 0.26, and 0.20 for HOL, RDC and JER, respectively, among candidate bulls born in 2022 (Table 2).

There has been a positive genetic trend for Saved Feed among bulls with genotype information between 2010 and 2023 with an average increase of 4 Saved Feed index units over the past decade (Figure 1).

The correlations between predicted breeding values for Saved Feed and DMI were moderately high and negative (-0.55 to -0.75), which means that selection for enhanced Saved Feed applies selection pressure against feed intake (Table 2). The correlations between predicted breeding values for Saved Feed and the Yield index were low to moderate (0.07 to 0.26), which indicates that feed efficient cows tend to produce more solids and less fluids. The correlations between predicted breeding values for Saved Feed and Female fertility, Udder health, and General health were low and ranged from -0.14 to 0.12 (Table 2). HOL and RDC had moderately negative correlations between predicted breeding values for Saved Feed and frame size (-0.19 to -0.29), whereas for JER this correlation was approximately zero. For JER, the correlation between predicted breeding values for Saved Feed and Longevity was slightly negative (-0.09; Table 2). Lastly, the correlations between predicted breeding values for

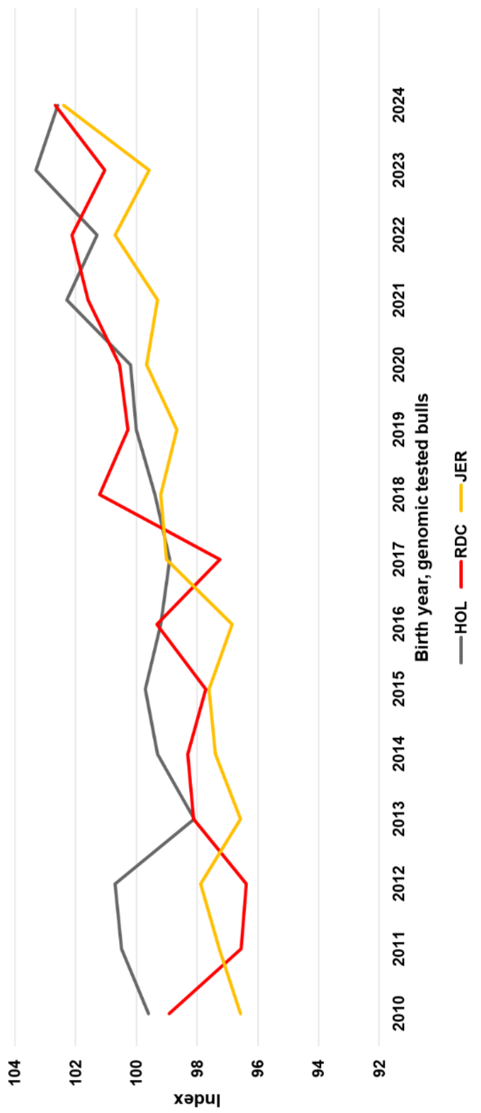

Figure 1: Genetic trends for Saved Feed among bulls with genotype information from Holstein (HOL), Red Dairy Cattle (RDC), and Jersey (JER).

Longevity and BW were low and negative in JER and HOL (Table 3).

Table 2: Index correlations for 2022 candidate bulls from Saved Feed to other breeding goals traits.

	Saved Feed		
	HOL (3,267)	RDC (2,592)	JER (488)
DMI	-0.55	-0.75	-0.55
NTM	0.30	0.26	0.20
Yield	0.26	0.07	0.23
FERT	-0.02	0.12	-0.14
MAST	-0.11	0.06	-0.11
GH	-0.11	-0.14	-0.08
Frame	-0.19	-0.29	0.01
Udder	-0.15	-0.08	-0.22
YSS	0.11	0.14	NA
LONG	0.01	0.17	-0.09

HOL=Holstein, RDC=Red Dairy Cattle, JER=Jersey, DMI=Dry Matter Intake, NTM=Nordic Total Merit, FERT=Female Fertility, MAST=Mastitis, GH=General Health, YSS=Young Stock Survival, LONG=Longevity, NA=Not available.

The slightly negative correlations between predicted breeding values for Saved Feed and functional traits may originate from the correlations between DMI and ECM, and the functional traits, since these have mostly negative correlations (Table 3) to female fertility (DMI: -0.05 to -0.30; ECM: -0.20 to -0.30), udder health (DMI: -0.05 to -0.15; ECM: -0.15 to -0.20), and general health (DMI: 0.00 to -0.15; ECM: -0.20 to -0.30).

Conclusions

The updated NAV Saved Feed evaluation has improved utilization of data, and it provides more enhanced feed efficiency selection indices to Nordic dairy farmers. The updated Saved Feed index has been fully weighed into the Nordic breeding goal NTM since February 2025. This has resulted in moderate correlations between the new NTM and the updated Saved Feed index which indicates that selection for NTM leads to genetic progress for feed efficient and profitable Nordic Dairy Cattle.

Table 3: Index correlations among 2022 candidate bulls between component traits for Saved Feed component traits and functional traits in the Nordic breeding goal.

	HOL			RDC			JER		
	DMI	ECM	BW	DMI	ECM	BW	DMI	ECM	BW
FERT	-0.10	-0.20	0.00	-0.30	-0.25	-0.25	-0.05	-0.30	0.00
MAST	-0.05	-0.15	0.05	-0.15	-0.15	0.00	-0.05	-0.20	0.00
GH	-0.15	-0.30	0.00	0.00	-0.20	0.00	-0.10	-0.20	0.00
YSS	-0.20	0.10	-0.20	-0.15	0.05	-0.20			
LONG	0.00	0.00	-0.20	-0.20	0.05	0.20	0.00	-0.10	-0.05

HOL=Holstein, RDC=Red Dairy Cattle, JER=Jersey, DMI=Dry Matter Intake, ECM=Energy Corrected Milk, BW=Body Weight, FERT=Female Fertility, MAST=Mastitis, GH=General Health, YSS=Young Stock Survival, LONG=Longevity.

Acknowledgments

The authors would like to acknowledge the research funding in this project: the CFIT project (9090-00083B, Innovation Fund Denmark). The authors wish to acknowledge former NAV director Gert Pedersen Aamand for his contributions and for leading the NAV Saved Feed group in the process of updating the NAV Saved Feed index.

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The M3GE project: developing beef cattle multi-trait multi-breed multi-country genomic evaluations for sustainability traits and small populations

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Abstract

Sustainability traits, such as feed efficiency and enteric methane emissions, are difficult and expensive to measure. Establishing a large national reference population is therefore challenging, and pooling data across countries in a joint international evaluation would be beneficial. In beef cattle, data on sustainability traits are collected across multiple breeds and in small populations, including crossbred animals of various breed composition. In such scenarios, genomic prediction requires modelling the individuals' different genetic background. Additionally, including available data on correlated indicator traits could improve the accuracy of genomic prediction for sustainability traits. However, current international beef cattle evaluations led by Interbeef are pedigree-based, performed within each breed separately, and use data from one trait, or one group of traits, at a time. The "M3GE" project aims to develop multi-trait multi-breed multi-country genomic evaluations for beef cattle, focusing on sustainability traits and small populations. The project is the result of a collaboration between WUR, ICAR (the Netherlands), Interbull Centre (Sweden), ICBF (Ireland), AHDB, SRUC (Great Britain), and FedANA (Italy), involving six national breeding organisations from three countries. The aims of this paper are to: i) present the M3GE project and its objectives, ii) give an overview of the status of collecting and modelling feed efficiency across participating organisations, and iii) present the first results of the project. Pedigree, phenotypic, and genomic data for feed efficiency, longevity, and associated indicator traits have been collected using the Interbull Centre's GenoEx-GDE and IDEA platforms. Initial work will focus on feed efficiency for which individual direct measures have been collected on ~13K phenotyped animals (~9K of which are genotyped), from over 15 different breeds and crossbreds recorded in Great Britain, Ireland, and Italy. The first steps include the imputation of collected genotypes to a common reference panel, population structure analysis, estimation of connectedness measures across populations, and estimation of genetic correlations across countries. The final step is to develop an international multi-breed single-step evaluation for feed efficiency including crossbred animals. This project contributes to the development of sustainable genomic evaluations in beef cattle for large and small populations.

Key words: beef cattle, international evaluations, multi-trait, multi-breed, genomic evaluations, feed efficiency

Introduction

Novel and sustainability traits, such as feed efficiency and enteric methane emissions, are difficult and expensive to measure, making it challenging to establish large reference populations at the national level. Hence, combining data across countries into a single international genomic evaluation is an attractive solution to build a large reference population for genomic predictions.

International beef cattle evaluations led by Interbeef involve up to fifteen countries worldwide and are carried out for five major popular breeds (Angus, Charolais, Hereford, Limousin, Simmental), and four trait groups: growth (composed by weaning weight), calving (composed by calving ease and birth weight), and carcass (composed by weight, conformation and fat) (Macedo, 2023; Venot et al., 2014; Vesela et al., 2019). Interbeef evaluations are performed within each breed separately and use data from one group of traits at a time. In Interbeef, raw national data (pedigree and phenotypes) are pooled at the international level and modelled using a pedigree-based evaluation following the AMACI model (Animal Model Accounting for Across Country Interactions) (Phocas et al., 2005). The resulting estimated breeding values (EBVs) and reliabilities (RELs) are distributed back to each participating country according to their national scale for a list of publishable sires (Bonifazi et al., 2023). Bonifazi et al. (2022) showed the feasibility and advantages of also including SNP genotypes at the international level to implement a joint single-breed single-step beef cattle genomic evaluation for a group of traits at a time.

In the Interbeef context, national beef cattle evaluations face different challenges that could be addressed through international collaboration. Firstly, the implementation of genetic evaluations for novel traits is highly desirable; however, the high costs associated with data recording hinder their widespread adoption. Consequently, genetic progress on new traits remains limited or is delayed, primarily due to the small size of the available reference populations at the national level. Second, small and local breeds risk becoming non-competitive in favour of more popular ones. Indeed, small and local breeds are not yet considered in current international, and sometimes even national, evaluations. Third, although crossbreeding is already widely used in some countries (e.g., Ireland) and is becoming more popular in others with the increased use of beef-on-dairy, international evaluations are still defined per breed and focus on purebred animals. In particular, in the presence of crossbred animals of various breed composition, genomic prediction requires modelling of the individual's different genetic background. Thus, there is a need to develop new and improved services for countries and to expand the current Interbeef portfolio to allow the inclusion of genomic data, new breeds and crossbreeds, and new (novel) traits.

We conducted a preliminary survey to identify which (novel) trait groups were of interest to Interbeef participating countries and which breeds already had data collected at the national level. Results revealed that feed efficiency and longevity were the most interesting trait groups to implement, alongside consideration of crossbred information (e.g., beef-on-dairy). Furthermore, the survey emphasised that pedigree, phenotypic, and genomic data are already available at the national level, but collected on different purebred and crossbred animals across different countries.

Based on the survey results, the project called "M3GE" (multi-trait multi-breed multi-country genomic evaluations) has been launched in 2024. This paper aims to: i) present the M3GE project and its objectives, ii) give an overview of the status of collecting and modelling feed efficiency across participating organisations, and iii) present the first results of the project.

The M3GE project

The M3GE project aims to develop a multi-trait multi-breed multi-country genomic evaluation for beef cattle, focusing on sustainability traits and small populations. Through such an evaluation, the project aims to unlock several potential advantages. First, including data on new purebred and crossbred animals would help expand the reference population for genomic prediction, potentially increasing the accuracy of genomic EBVs (GEBVs) and providing international GEBVs for both purebred and crossbred animals. Second, the evaluation would facilitate pooling data for new suitability traits at the international level, effectively harnessing collaboration among countries for novel and complex traits such as feed efficiency. Third, by involving new numerically small and local breeds, the project would increase the use of local genetic resources that are currently unexplored at the international level, as is the case for local transboundary breeds (local and genetically similar populations separated by national borders). Fourth, by leveraging the joint international reference population, the evaluation could allow countries to deliver GEBVs to their breeders for traits that are not yet evaluated at the national level due to the small size of their national reference population.

The M3GE project is a private-public-partnership (PPP) supported by the Dutch Ministry of Economic Affairs and is a collaboration between Wageningen University & Research (WUR), the International Committee for Animal Recording (ICAR), the Interbull Centre, the Irish Cattle Breeding Federation (ICBF), the Agriculture and Horticulture Development Board (AHDB), Scotland's Rural College (SRUC), and the National Federation of National Breeders Associations (FedANA). The project involves a total of six national breeding organisations from three countries: Ireland (IRL), Great Britain (GBR), and Italy (ITA). Next to ICBF (IRL), and AHDB and SRUC (GBR), there are four Italian national organisations involved in the project through FedANA: ANABIFJ (Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana), ANAPRI (Associazione Nazionale Allevatori Bovini di Razza Pezzata Rossa Italiana), ANABIC (Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane), ANACLI (Associazione Nazionale degli Allevatori delle razze bovine Charolaise e Limousine Italiane).

Materials and Methods

Data collection

Individual pedigree, phenotypic, and genomic information was collected at the Interbull Centre (Uppsala, Sweden). Pedigree and phenotypic repeated records were collected using IDEA, which was adapted to support the submission of repeated records. Genomic information was collected using GenoEx-GDE (Interbull Centre, 2025a). Data were collected for two new traits (feed and longevity) and their associated indicator traits. Therefore, two new trait groups were defined as follows:

- **FEED**, composed of feed itself (FEF) and its associated indicator traits: carcass traits (weight (CAW), fat (CAF), and conformation (CCO)), liveweight (LW), and growth traits (average daily gain (ADG) and average daily carcass weight (ACG)).
- **LONG**, composed of longevity (LON) and its associated indicator traits: calving traits (age at first calving (AFC), and calving interval (CAI)).

Data for FEED were collected for purebred beef animals, crossbred animals (both beef-on-beef and beef-on-dairy), and growing purebred dairy males, such as bulls and steers. Data for LONG were collected for purebred beef, crossbred (beef-on-beef and beef-on-dairy) animals.

Genomic information

Individual genomic information in the form of (imputed) single-nucleotide polymorphisms (SNPs) was available at medium-high density at the national level. Genomic information was collected for any animal with genomic and/or phenotypic information and their relatives (ancestors and sibs) to be later used in a single-step approach (Legarra et al., 2014). Different genotyping chips (i.e., panels) may be used within and across organisations. In total, eleven chips were collected among all participating organisations, with densities ranging from 30,111 to 777,962 SNPs. For each chip, a map file with information on the SNPs' commercial names, their physical positions on the genome (chromosome and base pair position), and their genome assembly version was collected. Chips and associated genomic information were validated to ensure that the collected genomic information followed the same Illumina AB coding (Illumina, 2006), and that genotype information would agree within- and across-country as well as within- and across-breed. Finally, all genotypes were mapped to the UMD3.1 genome assembly (Zimin et al., 2009).

Population structure

A Principal Component Analysis (PCA; Patterson et al., 2006; Chang et al., 2015) for FEF was performed using only SNPs overlapping across all chips (~4,000 SNPs) and including genotyped animals with phenotype and their genotyped ancestors. The PCA included about 18,000 genotyped animals, of which about 9,000 had a FEF phenotype.

Modelling of FEF at the national level

National information on FEF trait and model definitions, including (genetic) parameters, were collected using Interbeef 603 files (Interbull Centre, 2025b) and extended national genetic evaluations forms (Interbull Centre, 2025c).

Results & Discussion

Data collected

Table 1 shows an overview of the number of phenotypes and genotypes collected within the M3GE project for each organisation and trait group. Overall, data from GBR and IRL are collected on both purebred and crossbred animals, while data from ITA are collected only on purebred animals.

For the FEED trait group, about 98.5 thousand FEF repeated records have been collected across all organisations and breeds.

The number of records collected on FEED indicator traits ranges from 12.9 thousand for ADG to 12.9 million for CFA and CCO, with the majority of indicator traits' phenotypes being collected in IRL for carcass and live weights (Table 1). For FEED indicator traits, LW records are available in all organisations and countries, except for the Italian local breeds. Other indicator traits for FEED are only collected in either one or two countries. For the LONG trait group, approximately 10.7 million LON records have been collected from IRL and ITA, with the majority of phenotypes originating from the former. For LONG indicator traits, phenotypes have been collected for IRL and ITA with ~3.3 million and ~9.5 million records, respectively.

A total of about 3.1 million genotypes have been collected across organisations for both purebred and crossbred animals (Table 1). The majority of the genotypes are from IRL (~3 million), followed by GBR (~106 thousand), and ITA (~35 thousand).

Recording and modelling feed in different national organisations

Ireland

ICBF collects FEF at the Tully research station next to other novel phenotypes (e.g., enteric methane emissions). FEF is collected on commercial beef animals from targeted candidate sires, specifically the offspring of AI bulls, ensuring genetic connectedness to the rest of the IRL population. FEF recording is done close to the finishing period, mainly for steers and heifers, and a few young bulls. The age at recording ranges from about 200 to 900 days. While recording was initially focused on purebred animals, it is now mostly conducted on crossbred animals. Initially, when recording focused on purebred individuals, the diet was *ad libitum* (concentrate and hay), while nowadays is a TMR (Ryan et al., 2022). A single record

Table 1. Overview of the number of collected phenotypes (repeated records included) and genotypes [1].

Country	**GBR**	**IRL**	**ITA**				
Organization (breeds) [2]	AHDB&SRUC (PBD & XBD)	ICBF (PBD & XBD)	ANAFIBJ (HOL)	ANAPRI (SIM)	ANABIC (ITA breeds)	ANACLI (LIM & CHA)	
Trait [3]							**Total**
FEED							
FEF	28.4K	8.4K	8.8K	900	27K	24K	98.5K
CWE		12.9M				42K	13M
CFA		12.9M					12.9M
CCO		12.9M					12.9M
LW1	581K	1.0M	26K	7.6K		6K	1.6M
LW2		1.0M				192K	1.0M
LW3		1.3M					1.3M
ADG				7.6K	5.3K		12.9K
CDG					355K		355K
LONG							
LON		10.2M			248K	239K	10.7M
CAI		8.5M			912K		9.5M
AFC		3.1M			255K		3.3M
Genotypes	106K	3.0M	5.3K	3.3K	13.3K	12.9K	3.1M

[1] K = thousands, M = millions. [2] PBD = purebred, XBD = crossbred, HOL = Holstein-Friesian, SIM = Simmental, ITA breeds = Chianina, Marchigiana, Romagnola, Podolica, Maremmana, LIM = Limousin, CHA = Charolais. [3] FEED = feed trait group, FEF = feed, CWE = carcass weight, CFA = carcass fat, CCO = carcass conformation, LW1, LW2, LW3 = live weights (different definitions), ADG = average daily gain, CDG = carcass daily gain, LONG = longevity trait group, LON = longevity, CAI = calving interval, AFC = age at first calving.

per animal is available, with FEF defined as the average daily dry matter intake (Kg DMI/day) over the whole testing period. The minimum length of the testing period is 30 days and the average length is 109 days. The national model used is a multi-breed, multi-trait, two-step genomic animal evaluation including purebred and crossbred data for: FEF, three live weights (365-450 days, 450-550 days, 550-700 days), skeletal development, and three carcass traits (CWE, CFO, CCO). In this evaluation, FEF is modelled as:

$$FEF = hrbxb + hrbxd + dhrbxb + dhbxd + dfrac + afi + a2fi + a3fi + damage + dampar + byr + twin + hysfi + a + e,$$

where: *FEF* = daily DMI, *hrbxb* = heterosis beef x beef (covariate), *hrbxd* = heterosis beef x dairy (covariate), *dhrbxb* = dam heterosis beef x beef (covariate), *dhrbxd* = dam heterosis beef x dairy (covariate), *dfrac* = dam breed fraction (covariate), *afi*, *a2fi*, and *a3fi* = age at feeding fitted as a linear, quadratic, and cubic covariate, respectively, *damage* = age of the dam (covariate), *dampar* = parity of the dam (fixed), *byr* = birth year of the animal (fixed), *twin* = twinning (fixed), *hysfi* = hear-year-season (random), *a* = animal genetic effect (random), *e* = residual effect (random).

Great Britain

AHDB collects FEF data from research and mostly commercial farms, using the provided equipment and protocol. Recording is done over a 63-day period with animals having an *ad libitum* diet. FEF is collected during the testing period on purebred and crossbred steers with age at recording ranging from about 170 to 560 days. FEF is defined by SRUC as the average daily dry matter intake (kg DMI/day), with one record per week, for up to 7 weeks in total. At the national level, FEF is modelled using a single-trait, multi-breed, ssGBLUP repeatability animal model, defined as:

$$FEF = fdg + brl + aaf + lve + h13 + h23 + h34 + r12 + r13 + r23 + pe + a + e,$$

where: *FEF* = daily DMI, *fdg* = feeding group (fixed), *brl* = birth location (fixed), *aaf* = age at feeding (covariate), *lve* = live weight (covariate), *h13*, *h23*, *h34*, *r12*, *r13*, and *r23* = heterosis (*h*) and recombination (*r*) covariates between dairy breeds (1), beef breeds (2), continental breeds (3), and UK beef breeds (4), *pe* = animal permanent environment effect (random), *a* = animal genetic effect (random), *e* = residual effect (random).

Italy

In Italy, national breed organisations independently collect and model FEF recorded at their own test centre for (young) male selection candidates.

Holstein. ANAFIBJ collects FEF on purebred Holstein growing male selection candidates. The age at recording ranges from about 100 days to 600 days. Repeated records are collected over a minimum 30-day period. FEF is defined as daily dry matter intake (Kg DMI/day). FEF is modelled using a single-trait, genomic BLUP (GBLUP) repeatability animal model, defined as:

$$FEF = aaev + bidt + dtpt + pe + a + e,$$

where: *FEF* = daily DMI, *aave* = age at phenotyping (covariate), *bidt* = birth date (covariate), *dtpt* = date at phenotyping (random), *pe* = animal permanent environment effect (random), *a* = animal genetic effect (random), *e* = residual effect (random).

Simmental. ANAPRI collects FEF on purebred Simmental male selection candidates. The age at recording ranges between about 280 and 340 days. Repeated records are collected over a 60-day period and summarised into a single record per animal. FEF is defined as daily residual feed intake (RFI; Kg DMI/day). FEF is modelled using a single-trait, single-step GBLUP animal model as:

$$FEF = cg + weight + a + e,$$

where: FEF = daily RFI, cg = contemporary group (fixed), $weight$ = animal live weight (covariate), *a* = animal genetic effect (random), *e* = residual effect (random).

Italian local beef breeds, Limousin, and Charolais. ANABIC collects FEF on purebred male selection candidates for three Italian local beef cattle breeds: Chianina, Marchigiana, and Romagnola. FEF is available over a 30-day testing period, and the age at recording ranges between about 200 and 400 days. FEF is defined as residual feed intake (RFI; kg DMI/day). FEF data for the three local beef breeds are jointly modelled using a multi-breed, single-trait, ssGBLUP repeatability animal model as:

$$FEF = cg + breed + weight + pe + a + e,$$

where: FEF = daily RFI, cg = contemporary group (fixed), $breed$ = breed effect (fixed), *weight* = animal weight (covariate), *pe* = animal permanent environment effect (random), a = animal genetic effect (random), e = residual effect (random).

ANACLI collects FEF on purebred Limousin and Charolais male selection candidates at the ANABIC test centre using the same procedure. FEF is defined as residual feed intake (kg DMI/day). For FEF and Limousin individuals, a single-trait, ssGBLUP repeatability animal model was recently developed as:

$$FEF = cg + weight + pe + a + e,$$

where: FEF = daily RFI, cg = contemporary group (fixed), $weight$ = animal weight (covariate), pe = animal permanent environment effect (random), a = animal genetic effect (random), e = residual effect (random). The FEF data from ANACLI was not included in the PCA as it was not yet available.

Table 2 shows a summary of the national trait definitions of FEF, evaluations and model used, and genetic parameters estimated at the national level by the different organisations. Overall, FEF heritabilities are moderate (average across organisations of 0.22), ranging between a minimum of 0.05 for the Italian local beef breeds and 0.32 for IRL. Repeatability ranged between 0.22 for the Italian local beef breeds and 0.50 for the Italian Holstein.

Population structure

The first two principal components (PC) explained a large proportion of variance (24.4 % and 15.3%, respectively). Figure 1 shows the PCA for purebred individuals. The first PC separated purebred individuals into several main breed clusters independent of the country providing the genotypes: from left to right, Holstein, Limousin, Angus, and Simmental. The local Italian breeds clustered together and separately from other purebred individuals following the second PC. The remaining purebred individuals clustered closely with either the Limousin or the Angus breed cluster. Finally, Figure 2 shows the PCA including crossbred animals, demonstrating how crossbred individuals form a continuum across purebred animals, except for the local Italian breeds, which remain distinct as they were not crossed with other breeds. Such a pattern was expected as the majority of the crossbred animals are from unstructured crosses.

Table 2. Summary of national models and genetic parameters for the feed trait group across different countries.

Country	**GBR**		**IRL**		**ITA**	
Organization	AHDB&SRUC	ICBF	ANAFIBJ	ANAPRI	ANABIC	ANACLI
Breeds[1]	PBD & XBD	PBD & XBD	HOL	SIM	ITA breeds	LIM & CHA
Trait[2]	DMI	DMI	DMI	RFI	RFI	RFI
Model[3]	ssGBLUP multi-breed single-trait	two-step genomic multi-breed multi-trait	GBLUP single-trait	ssGBLUP single-trait	ssGBLUP multi-breed single-trait	ssGBLUP single-trait
Heritability	0.14	0.30	0.32	0.29	0.05	0.13
Repeatability	0.26		0.50		0.22	0.24

[1] PBD = purebred, XBD = crossbred, HOL = Holstein-Friesian, SIM = Simmental, ITA breeds = Chianina, Marchigiana, Romagnola, LIM = Limousin, CHA = Charolais. [2] DMI = Dry Matter Intake, RFI = Residual Feed Intake. [3] ssGBLUP = single-step genomic BLUP, GBLUP = genomic BLUP.

Next steps and implications

The next steps in the project are to impute the collected genotypes to a common reference panel, and to estimate connectedness and genetic correlations across populations and breeds. Then, an international multi-breed single-step evaluation for FEF, including crossbred animals, will be developed. Later, the project will focus on developing similar multi-breed single-step evaluations for LON. In the final phase, the project will focus on including indicator traits for both FEF and LON using a multi-trait approach.

The M3GE project is expected to improve current and future Interbeef evaluations by optimising existing services and developing new ones. Such improvements include, for instance, adapting pipelines to accommodate repeated records as well as identifying possible bottlenecks, such as the efficient upload and routine handling of large volumes of genomic data from individuals with diverse breed composition. Overall, the M3GE project contributes to the development of sustainable international genomic evaluations in beef cattle across both large and small populations, thereby enhancing Interbeef's capacity to meet future demands.

Conclusions

The M3GE project is an international collaboration between different partners, including six national breeding organisations. The project aims to develop beef cattle multi-trait multi-breed multi-country genomic evaluations for sustainability traits and small populations. In the first phase, data (pedigree, phenotypes, genotypes) have been collected for feed, longevity, and their associated indicator traits, for both purebred and crossbred animals, including (small) local populations. The next step is to develop a multi-breed, multi-country genomic evaluation for feed efficiency.

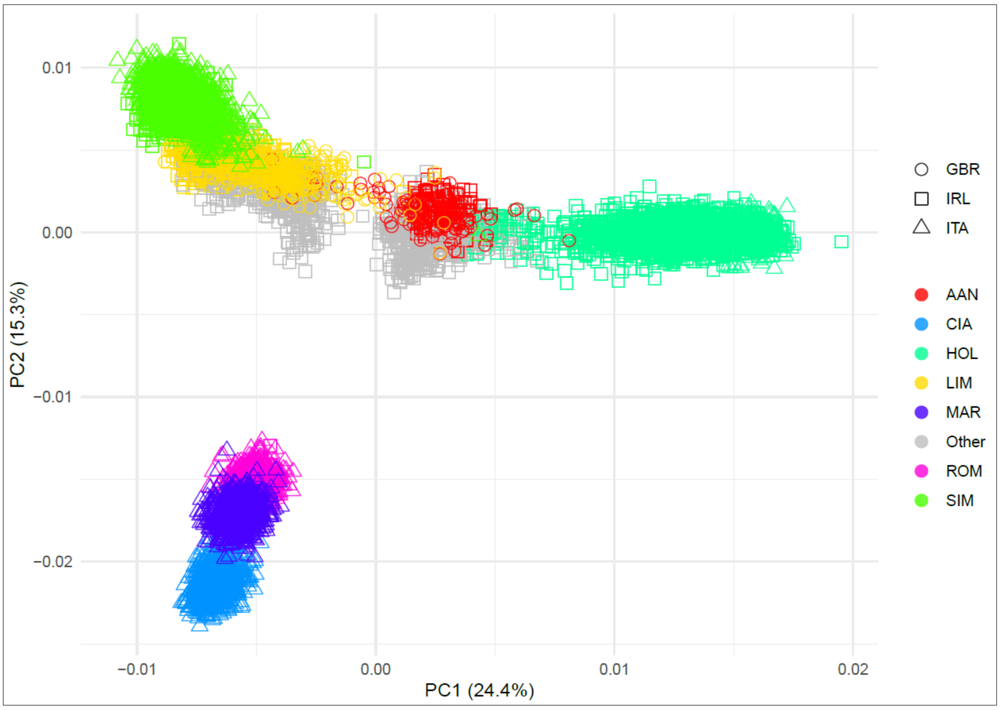

Figure 1. Plot of the first two principal components (PC) and percentage of explained variance (within brackets) of the genomic relationship matrix for purebred animals. Shapes indicate the country sending the genotype and colours indicate the breed (Other = other purebred breeds). GBR = Great Britain, IRL = Ireland, ITA = Italy.

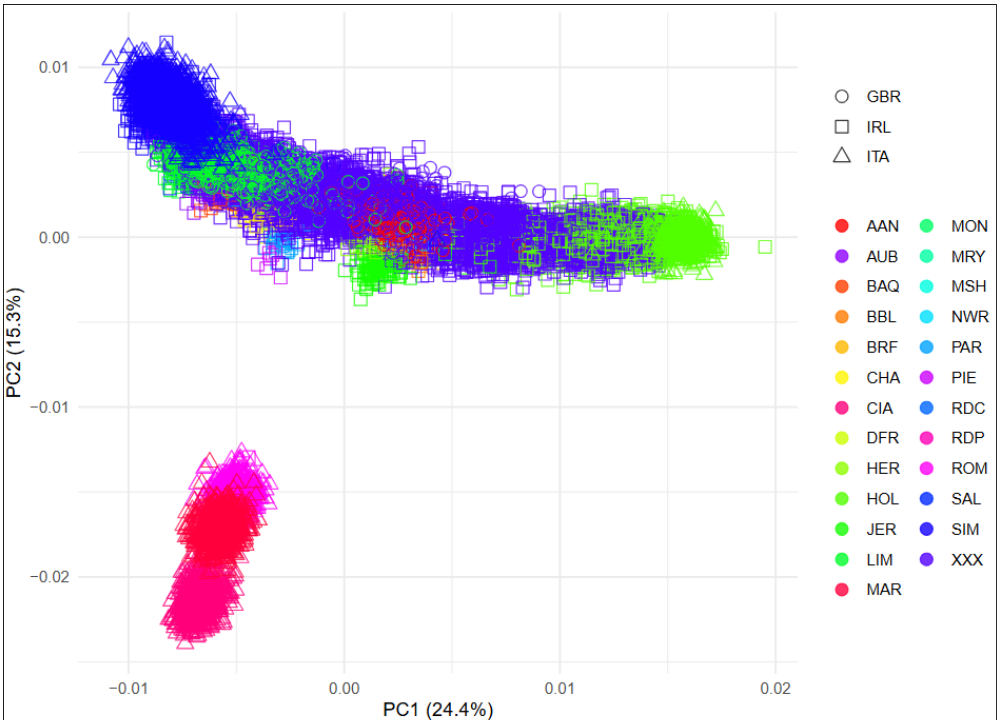

Figure 2. Plot of the first two principal components (PC) and percentage of explained variance (within brackets) of the genomic relationship matrix for purebred and crossbred animals. Shapes indicate the country sending the genotype and colours indicate the breed. GBR = Great Britain, IRL = Ireland, ITA = Italy.

Acknowledgments

This study was financially supported by the Dutch Ministry of Economic Affairs (TKI Agri & Food Project LWN23062) and the M3GE partners ICAR (Utrecht, the Netherlands), SLU (Uppsala, Sweden), AHDB (Coventry, United Kingdom), SRUC (Scotland, United Kingdom), FedANA (Rome, Italy), and ICBF (Cork, Ireland).

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