

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-GEV 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 CH₄ 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_{EF} sub-index, leading to Residual Methane Efficiency Functional (RMEF);
3. Relative to all traits, using the Walloon V_{EG} 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 h^2 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.

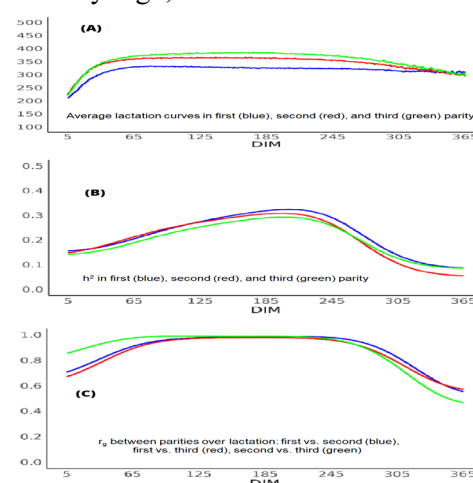


Figure 1. (A) Lactation curves for methane emissions (PCH4) in first (blue), second (red), and third (green) parity, (B) Heritability of PCH4 across lactation for each parity, and (C) genetic correlation across parities across the lactation.

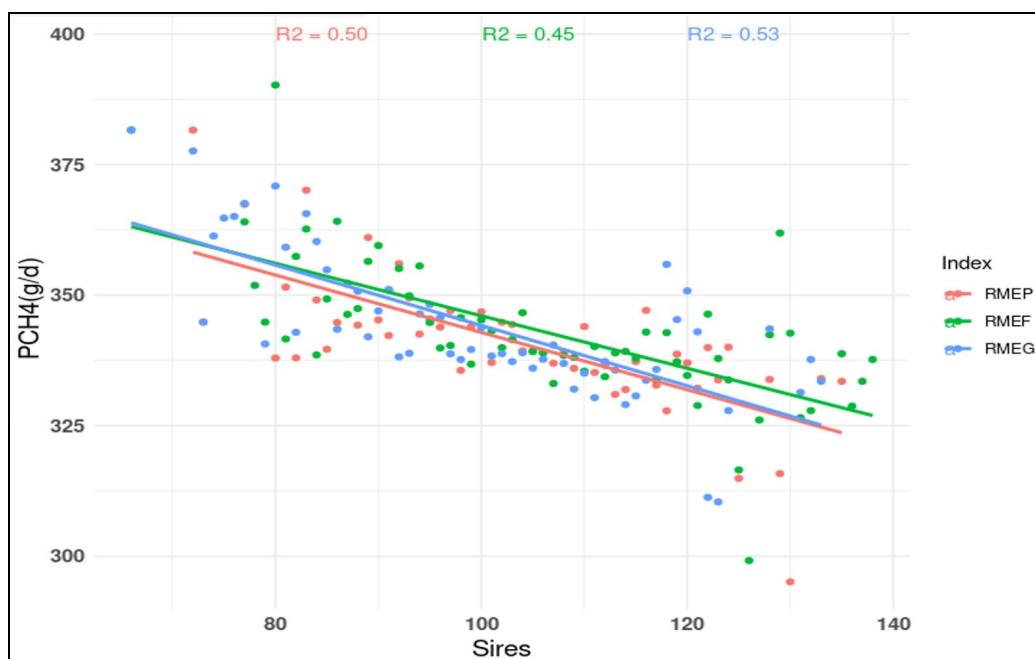


Figure 2. Distribution of average methane emissions (PCH4) for the 1020 daughter groups, sires sorted according to their relative GEBV, the consistency of the impact of selection being reported through R^2 values for RMEP, RMEF and RMEG.

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.

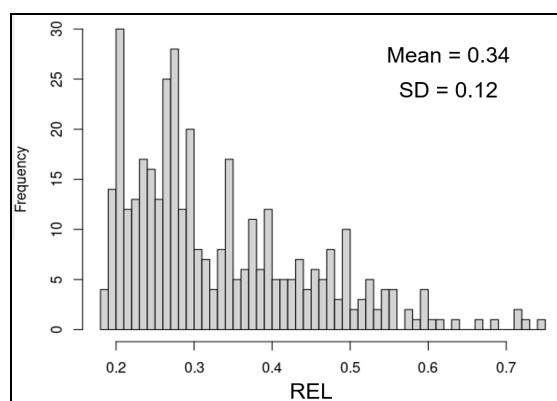


Figure 3. Distribution of reliability (REL) of common sires from Country A

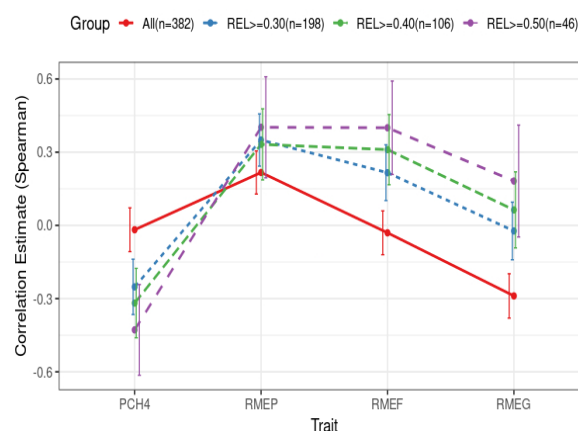


Figure 4. Visualization of the Blanchard et al. (1983) adjusted rank correlation based on common sires from Country A.

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.

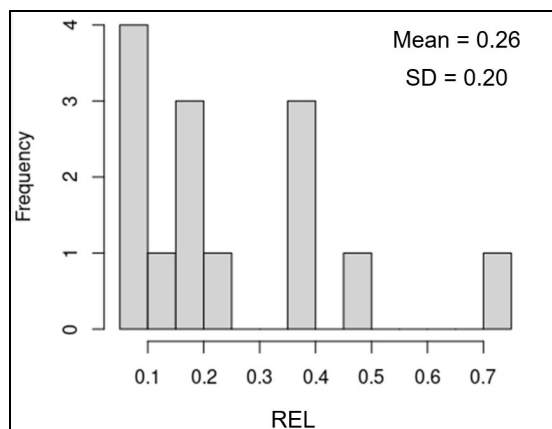


Figure 5. Distribution of reliability (REL) of common sires from Country B

As shown in Figure 5 the mean level of REL in the common bulls is extremely low.

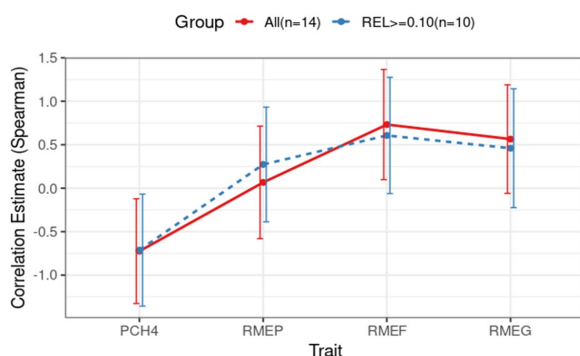


Figure 6. Visualization of the Blanchard et al. (1983) adjusted rank correlation based on common sires from Country B.

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 VEG 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 VEG which one can be considered the most efficient.

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References

- Ben Zaabza, H., Taskinen, M., Mäntysaari, E.A., Pitkänen, T., Pedersen Aamand, G., and Strandén, I., 2022. Breeding value reliabilities for multiple-trait single-step genomic best linear unbiased predictor. *J. Dairy Sci.* 105 (6), 5221–5237. <https://doi.org/10.3168/jds.2021-21016>
- Blanchard, P.J., Everett, R.W., and Searle, S.R., 1983. Estimation of genetic trends and correlations for Jersey cattle. *J. Dairy Sci.* 66 (9), 1947–1954. [https://doi.org/10.3168/jds.S0022-0302\(83\)82033-5](https://doi.org/10.3168/jds.S0022-0302(83)82033-5)
- Gao, H., Kudinov, A.A., Taskinen, M., Pitkänen, T.J., Lidauer, M.H., Mäntysaari, E.A., and Strandén, I., 2023. A computationally efficient method for approximating reliabilities in large-scale single-step genomic prediction. *Genet. Sel. Evol.* 55, 1. <https://doi.org/10.1186/s12711-022-00774-y>
- Garnsworthy, P.C., G.F. Difford, G.F., M.J. Bell, M.J., A.R. Bayat, A.R., P. Huhtanen, P., B. Kuhla, B., J. Lassen, J., N. Peiren, N., M. Pszczola, M., D. Sorg, D., M.H.P.W. Visker, M.H.P.W., and T. Yan. T. 2019. Comparison of methods to measure methane for use in genetic evaluation of dairy cattle. *Animals* 9 (10), 837. <https://doi.org/10.3390/ani9100837>
- Grelet, C., Fernández Pierna, J.A., Dardenne, P., Baeten, V., and Dehareng, F. 2015. Standardization of milk mid-infrared spectra from a European dairy network. *J. Dairy Sci.* 98 (4), 2150–2160. <https://doi.org/10.3168/jds.2014-8764>
- Vanlierde, A., Dehareng, F., Gengler, N., Froidmont, E., McParland, S., Kreuzer, M., Bell, M., Lund, P., Martin, C., Kuhla, B., and H. Soyeurt. 2021. Improving robustness and accuracy of predicted daily methane emissions of dairy cows using milk mid - infrared spectra. *J. Sci. Food Agric.* 101 (8), 3394–3403. <https://doi.org/10.1002/jsfa.10969>