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Multitrait genomic prediction of methane emissions in Danish Holstein cattle

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ABSTRACT

In dairy cattle, selecting for lower methane-emitting animals is one of the new challenges of this decade. However, genetic selection requires a large number of animals with records to get accurate estimated breeding values (EBV). Given that CH_4 records are scarce, the use of information on routinely recorded and highly correlated traits with CH_4 has been suggested to increase the accuracy of genomic EBV (GEBV) through multitrait (genomic) prediction. Therefore, the objective of this study was to evaluate accuracies of prediction of GEBV for CH_4 by including or omitting CH_4, energy-corrected milk (ECM), and body weight (BW) as well as genotypic information in multitrait analyses across 2 methods: BLUP and single-step genomic BLUP (SSGBLUP). A total of 2,725 cows with CH_4 concentration in breath (14,125 records), BW (61,667 records), and ECM (61,610 records) were included in the analyses. Approximately 2,000 of these cows were genotyped or imputed to 50K. Ten cross-validation groups were formed by randomly grouping paternal half-sibs. Five scenarios were performed: (1) base scenario with only CH_4 information; (2) without CH_4, but with information from BW, ECM, or BW+ECM only in reference population; (3) without CH_4, but with information from BW, ECM, or BW+ECM in both validation and reference population; (4) with CH_4 information and BW, ECM, or BW+ECM information only in the reference population; and (5) with CH_4 information and BW, ECM, or BW+ECM information in both validation and reference population. As a result, for each method (BLUP, SSGBLUP), 13 sub-scenarios were performed, 1 from scenario 1, and 3 for each of the subsequent 4 scenarios. The average accuracy of GEBV for CH_4 in the base scenario was 0.32 for BLUP and 0.42 for SSGBLUP, and it ranged from 0.10 in scenario 2 to 0.78 in scenario 5 across methods. In terms of bias, the base scenario 1 was unbiased for SSGBLUP; similar results were achieved with scenario 5. Including information on ECM increased the accuracy of GEBV for CH_4 by up to 61%, whereas adding information on both traits (BW and ECM) increased the accuracy by up to 90%. Scenarios that did not include CH_4 in the reference population had the lowest correlations (0.17–0.33) with single-trait CH_4 GEBV, and scenarios with CH_4 in the reference population had the highest correlations (0.41–0.81). Thus, failure to include CH_4 in future reference populations results in predicted CH_4 GEBV, which cannot be used in practical selection. Therefore, recording CH_4 in more animals remains a priority. Finally, multiple-trait genomic prediction using routinely recorded BW and ECM leads to higher prediction accuracies than traditional single-trait genomic prediction for CH_4 and is a viable solution for increasing the accuracies of GEBV for scarcely recorded CH_4 in practice.
Key words: multitrait genomic prediction, predictor trait, methane concentration

INTRODUCTION

Methane emission of dairy cattle represents 18% of the global greenhouse gas emissions (Knapp et al., 2014). This has led to a large number of research projects investigating opportunities to reduce methane emissions in dairy cattle (de Haas et al., 2011; Waghorn and Hegarty, 2011; Garnsworthy et al., 2012; Ross et al., 2013). From the genetic point of view, methane emission has shown to be a heritable trait (0.100.30; van Engelen et al., 2015; Lassen and Løvendahl, 2016; Pszczola et al., 2017; Breider et al., 2018; Difford et al., 2020), making it possible to select for lower emitting animals, with the advantage that genetic progress is cumulative and permanent. However, as CH_4 is a scarcely recorded trait, it would require a considerable number of cows with CH_4 records in the reference population to estimate genetic EBV (**GEBV**) of bulls with good accuracies (Hayes et al., 2009). At present, the accuracies of GEBV are low due to the limited number of cows with CH_4 records. One approach to increase the accuracy of prediction of GEBV for CH_4 given the limited amount of data available could be to in-

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clude information on routinely recorded traits that are highly genetically correlated with CH_4 in a multitrait genomic prediction. Before the genomic era, Ducrocq (1994) stated the benefits from multitrait prediction in 3 points: (1) the exploitation of indirect information provided by correlated traits that is not available in selection candidates, (2) the use of additional knowledge that can increase the accuracy of the genetic evaluations, and (3) the reduction of systematic biases in the evaluations. Likewise, Ducrocq (1994) reported increases in genetic gain when the genetic correlation between traits is high when (1) the difference between the genetic and the residual correlation is large; (2) the difference in heritability is large, and the goal trait is the one with lowest heritability; and (3) more than one random effect is considered, and the full- or half-sib family size is small. More recently, it has been suggested that multiple-trait genomic selection could lead to higher prediction accuracy than single-trait genomic selection (Calus and Veerkamp, 2011; Jia and Jannink, 2012), where the magnitude of genetic correlations between the traits is the key factor determining the increase in accuracy. Within this context, BW and ECM, traits that are routinely recorded in some automated milking robots and highly correlated with CH_4, could be good candidate predictor traits for CH_4.

To determine the benefit of including genotypic information in the prediction of the EBV and the consistency of the predictor traits across methods, 2 methods were tested. First, BLUP used pedigree-derived additive genetic relationships to estimate an EBV for each animal in the pedigree. Second, single-step genomic BLUP (**SSGBLUP**; Aguilar et al., 2010; Christensen and Lund, 2010) allowed the addition of phenotypic information of non-genotyped animals into the genomic BLUP method by combining in a single step the genomic relationship matrix (**GRM**) with the pedigree relationship matrix into a new relationship matrix, whose inverse is used to solve the mixed-model equations to obtain the GEBV. Therefore, the objective of this study is to evaluate the accuracy of prediction and bias of GEBV for CH_4 by testing a variety of scenarios with the presence or absence of predictor traits (ECM and BW) and genotypes.

MATERIALS AND METHODS

Phenotypes

A total of 2,725 Danish Holstein cows with CH_4 breath concentration (referred to as CH_4; 14,125 records), BW (61,667 records), and ECM (61,610 records) were available from the Danish Cattle Research Center (Foulum, Denmark; 1,328 cows) and 10 commercial farms (1,397 cows) in Denmark. The records were collected between 2011 and 2016 as described previously in Zetouni et al. (2018) and Difford et al. (2020). Methane data (from research and commercial herds) were filtered to include only weekly averages comprising 4 or more days of recording for each week of measurement and cows with a minimum of 3 repeated weekly measurements. Methane concentration was measured by 2 sniffer methods (Garnsworthy et al., 2012; Lassen et al., 2012): the nondispersive infrared CH_4 sensor (Guardian NG, Edinburgh Instruments Ltd., Livingston, UK) in the research farms and the portable Fourier transform infrared Gasmet DX-4000 (Gasmet Technologies Oy, Helsinki, Finland) in the commercial farms. The concordance correlation coefficient between both sniffers when measuring CH_4 concentration was 0.79 (Garnsworthy et al., 2019). Both methodologies were described and compared previously (Difford et al., 2016), where it was concluded that both instruments can be used interchangeably after calibration and standardization. As CH_4 concentration (in parts per million) was not normally distributed, a natural logarithm (ln) transformation was used, and the ln was multiplied by 100 to avoid problems with the scale of the other traits. The phenotypic correlation between CH_4 concentration and CH_4 in grams per day based on a previous study on the same data was 0.85, whereas the genetic correlation was 0.74 (C. I. V. Manzanilla-Pech, unpublished data). However, in this study, only the CH_4 concentration trait was used to avoid artificially induced covariation between traits in multitrait estimators, as estimated CH_4 in grams per day is a linear combination of the ratio CH_4:CO_2 concentration and the predictor traits ECM and BW (Madsen et al., 2010).

Weekly average records on BW and milk yield (**MY**) were collected between 1992 and 2016. Cows were located at Ammitsbøl Skovgaard research herd (Skovgaard, Vejle, Denmark) until 2000 and were subsequently relocated to the Danish Cattle Research Center in 2001 to 2016 as reported in Li et al. (2017). Cows were part of numerous nutritional experiments and diets that included primarily rolled barley, corn silage, grass clover silage, rapeseed meal, and soybean meal. The research barn Ammitsbøl Skovgaard was a tiestall system with twice-daily milking and sampling to measure milk quantity and components (fat and protein content). The Danish Cattle Research Center barn is a loose housing system with access to automatic milking systems (DeLaval International AB, Tumba, Sweden). Milk composition was determined using infrared technology at Eurofins (Vejen, Denmark) using CombiFoss equipment (Foss, Hillerød, Denmark). The automatic milking system was fitted with a weighing platform (Danvaegt, Hinnerup, Denmark) that recorded BW at

each milking from which weekly averages were calculated (full description can be found in Li et al., 2017). For the 10 commercial farms, weekly average MY and milk components were available by the national recording scheme (RYK, Skejby, Denmark).

Energy-corrected milk was calculated using the following equation (Sjaunja et al., 1990):

$$\text{ECM (kg)} = 0.25\text{milk (kg)} + 12.2\text{fat (kg)} + 7.7\text{protein (kg)}. \quad [1]$$

Genotypes and Pedigree

Two sets of genotypes were available; one set (1,747 cows) was genotyped with 50k Illumina Bovine SNP50 (Illumina, San Diego, CA), and the other set (466 cows) was genotyped with EuroGenomics 10K LD chip (EuroGenomics, Amsterdam, the Netherlands). The genotypes were edited for quality control with Plink software (Purcell et al., 2007). Quality control included a minimum of 0.02 for minor allele frequency, a maximum of 10% genotypes per SNP missing, a maximum of 15% genotypes per animal missing, and Hardy-Weinberg disequilibrium significant at $P = 0.001$. In addition, animals with duplicated genotypes, sex chromosome SNP, unmapped SNP, and SNP with duplicate or uncertain positions were deleted. Posteriorly, the LD chip genotypes were imputed to 50K with FImpute software (Sargolzaei et al., 2014). After editing and removing duplicates, 1,962 cows with 38,253 SNP remained. The full pedigree contained the identification of the cow, sire, and dam for around 49,000 individuals. After pruning for noninformative animals, 25,701 animals remained. The cows with phenotypes could trace back ancestors on average 9 generations in the pruned pedigree.

Variance Components and GEBV Estimation

Variance components for CH_4, BW, and ECM were estimated using the AI-REML algorithm with DMU software (Version 6, Release 5.4; Madsen and Jensen, 2014). Genetic and phenotypic correlations used for the GEBV calculation were estimated through multivariate analysis between the traits in the full population using pedigree information.

To test the change in accuracies by including genotypic information, 2 methods were used: BLUP with only pedigree information and SSGBLUP with genotypes plus pedigree information. The GEBV for CH_4 of each animal (through different scenarios and methods) were estimated using DMU (Madsen and Jensen, 2014) with BLUP and SSGBLUP. Both methods were implemented considering the same fixed effects and nongenetic random effects as in Equation 2. All SNP that passed quality control were used to calculate GRM according to VanRaden (2008), using the invgmatrix program included in DMU (Madsen and Jensen, 2014) together with the pedigree.

The BLUP and SSGBLUP in matrix notation with variance components estimated in Equation 2 and the inverse of relationship matrix A and H, respectively, are:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1a} + \mathbf{Z_2c} + \mathbf{e}, \quad [2]$$

where $\mathbf{y}$ is the vector of phenotypes; $\mathbf{b}$ represents the vector of fixed effects [herd, trial, year, season; lactation week modeled with the Wilmink function; type of sniffer (2 levels); and parity number as 1, 2, and 3+]; $\mathbf{X}$ is the incidence matrix relating observations with fixed effects; $\mathbf{a}$ is the vector of direct additive genetic effects; $\mathbf{Z_1}$ is the incidence matrix relating observations with random genetic effects; $\mathbf{c}$ is the vector of permanent environmental effects; $\mathbf{Z_2}$ is the incidence matrix relating observations with random permanent environmental effect; and $\mathbf{e}$ is the vector of residual effects. The models for BW and ECM were similar to Equation 2 but excluding the type of sniffer (Guardian or Gasmet) fixed effect. Distributions of the random effects are $\text{var}(\mathbf{a}) = \mathbf{A}\sigma_a^2$ for the BLUP method using only pedigree, where $\mathbf{A}$ is the pedigree relationship matrix and σ_a^2 is the additive genetic variance; $\text{var}(\mathbf{a}) = \mathbf{H}\sigma_a^2$ for the SSGBLUP method, where $\mathbf{H}$ is the combined pedigree and genomic relationship matrix and σ_a^2 is the additive genomic variance; $\text{var}(\mathbf{c}) = \mathbf{I}\sigma_c^2$, where $\mathbf{I}$ is the identity matrix of order equal to the number of individuals with records and σ_c^2 is the permanent environmental variance; and $\text{var}(\mathbf{e}) = \mathbf{I}\sigma_e^2$, where $\mathbf{I}$ is an identity matrix of an order equal to the number of observations and σ_e^2 is the residual variance. The inverse of the H-matrix, $\mathbf{H}^{-1}$, was calculated with the following equation (Aguilar et al., 2010; Christensen and Lund, 2010):

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \lambda\left(\mathbf{G}w^{-1} - \mathbf{A}_{22}^{-1}\right) \end{bmatrix}, \quad [3]$$

where $\mathbf{A}^{-1}$ is the inverse of the pedigree relationship matrix; λ is the value of 0.95 for lambda, $\mathbf{G}^{-1}$ is the inverse of the GRM, w is the relative weight on the polygenic effect, and $\mathbf{A}_{22}^{-1}$ is the inverse of the pedigree relationship matrix among genotyped animals.

Scenarios and Sub-Scenarios

Table 1 illustrates all the scenarios, sub-scenarios, and (co)variances used for each scenario. Thirteen sub-scenarios across 5 scenarios were performed, first a base single-trait scenario (1, CH_4), where only CH_4 information was considered. From scenario 2 to 5, each scenario has 3 sub-scenarios depending on the predictor trait included: (a) BW, (b) ECM, and (c) BW+ECM. Thus, the second scenario assumes the absence of CH_4 information, but with information available on predictor traits in the reference population: (2a) BW_OR, (2b) ECM_OR, and (2c) BW+ECM_OR. The third scenario assumes the absence of CH_4 information, but with information available on predictor traits in validation and reference population [(3a) BW_VR, (3b) ECM_VR, (3c) BW+ECM_VR]. The fourth scenario assumes the presence of information on CH_4 and predictor traits on the reference population [(4a) CH_4+BW_OR, (4b) CH_4+ECM_OR, (4c) CH_4+BW+ECM_OR]. Finally, the fifth scenario assumes the presence of information of CH_4 in the reference population and predictor traits in both validation and reference population [(5a) CH_4+BW_VR, (5b) CH_4+ECM_VR, (5c) CH_4+BW+ECM_VR].

Cross-Validation Groups

All 13 sub-scenarios across the 2 methods (BLUP and SSGBLUP) were validated using cross-validation, where the population of genotyped individuals was divided into 10 subsets, ensuring all paternal half-sibs were in the same group. The assignment to the groups was made by sire, using stratified random sampling, which was undertaken in 2 steps. First, the sires of genotyped animals were ranked from the highest to the lowest by number of daughters with CH_4 records. Then, from every set of 10 subsequent sires, 1 sire was

Table 1. Different scenarios and sub-scenarios performed per method (BLUP, SSGBLUP)[1]

Scenario description	Sub-scenario abbreviation[2]	Type of analysis	(Co)variances used to obtain GEBV[3]	Validation trait	Information included in the validation population	Information included in reference population
1. Base	1. CH_4	Univariate	Var CH_4	CH_4	—	CH_4
2. No CH_4, predictor traits only in reference	2a. BW	Bivariate	Var CH_4, Var BW, Cov CH_4-BW	CH_4	—	BW
	2b. ECM	Bivariate	Var CH_4, Var ECM, Cov CH_4-ECM			ECM
	2c. BW + ECM	Trivariate	Var CH_4, Var BW, Var ECM, Cov CH_4-BW Cov CH_4-ECM, Cov BW-ECM			BW and ECM
3. No CH_4, predictor traits on validation and reference	3a. BW	Bivariate	Var CH_4, Var BW, Cov CH_4-BW	CH_4	BW	BW
	3b. ECM	Bivariate	Var CH_4, Var ECM, Cov CH_4-ECM		ECM	ECM
	3c. BW + ECM	Trivariate	Var CH_4, Var BW, Var ECM, Cov CH_4-BW Cov CH_4-ECM, Cov BW-ECM		BW and ECM	BW and ECM
4. CH_4, predictor traits only reference	4a. CH_4 + BW_OR	Bivariate	Var CH_4, Var BW, Cov CH_4-BW	CH_4	—	CH_4 and BW
	4b. CH_4 + ECM_OR	Bivariate	Var CH_4, Var ECM, Cov CH_4-ECM			CH_4 and ECM
	4c. CH_4 + BW + ECM_OR	Trivariate	Var CH_4, Var BW, Var ECM, Cov CH_4-BW Cov CH_4-ECM, Cov BW-ECM			CH_4, BW, and ECM
5. CH_4, predictor trait on validation + reference	5a. CH_4 + BW_VR	Bivariate	Var CH_4, Var BW, Cov CH_4-BW	CH_4	BW	CH_4 and BW
	5b. CH_4 + ECM_VR	Bivariate	Var CH_4, Var ECM, Cov CH_4-ECM		ECM	CH_4 and ECM
	5c. CH_4 + BW + ECM_VR	Trivariate	Var CH_4, Var BW, Var ECM, Cov CH_4-BW Cov CH_4-ECM, Cov BW-ECM		BW and ECM	CH_4, BW, and ECM

[1]SSGBLUP = single-step genomic BLUP.
[2]CH_4 = methane concentration; OR = only reference; VR = validation + reference.
[3]GEBV = genomic EBV.

Table 2. Numbers of sires, cows in validation and reference populations, and CH_4, BW, and ECM records in reference population per cross-validation group

Population	Cross-validation group									
	1	2	3	4	5	6	7	8	9	10
Sires per group	57	57	57	56	56	57	57	57	56	56
Cows in validation	226	233	206	202	233	222	197	209	220	213
Cows in reference	2,004	1,997	2,024	2,028	1,997	2,008	2,033	2,021	2,010	2,017
CH_4 records in reference	12,800	12,652	13,414	12,946	12,843	12,158	13,393	12,927	12,724	12,704
BW records in reference	57,550	56,089	56,737	56,259	59,248	55,062	56,212	57,437	58,589	55,850
ECM records in reference	57,592	56,194	56,837	56,333	59,287	55,251	55,997	57,541	58,707	55,963

randomly allocated to 1 of the 10 groups. Thus, the 10 validation groups were similar in terms of the proportion of sires, number of cows with genotypes and the total number of records. The number of sires and cows in the cross-validation groups is shown in Table 2. The average number of cows per sire was 4, and the total number of sires was 566. For each of the validation groups, GEBV for CH_4 were predicted after excluding the respective CH_4 phenotypes from the analysis, using phenotypes of the other 9 groups as the reference population.

Accuracy and Bias Calculation

Adjusted phenotypes for CH_4 were calculated as the sum of the solutions per animal for genetic effects and permanent environmental effects with the full database, using all fixed effects in Equation 2. In this way, a unique phenotype per animal closer to the true phenotype was available instead of multiple true phenotypes. The accuracies were calculated via cross-validation per sub-scenario as the correlation between the adjusted phenotype for CH_4 and the GEBV for CH_4 divided by the formula adapted from Mrode (2013) computed to calculate the accuracy for repeated records:

$$\text{Accuracy} = \frac{r}{\sqrt{\frac{n\text{h}^2}{1+(n-1)t}}}, \qquad [4]$$

where r is the correlation between the adjusted phenotype and the GEBV, n is the average number of repeated records per animal per cross-validation group (6); h^2 is the heritability of the CH_4 (0.14 ± 0.05; see Results and Discussion); and t is the repeatability of CH_4 calculated as sum of the genetic and permanent environmental variances divided by the phenotypic variance (0.51). Accuracies were averaged across the 10 validation groups. Standard errors of accuracies per scenario were defined as the standard deviation of the accuracy across all validation groups divided by the square root of the number of validation groups (10). Slopes of regression (linear regression coefficients) of the adjusted phenotypes on the GEBV were calculated per method and per sub-scenario as a measure of the bias in terms of the variance in GEBV. Additionally, average pairwise comparison correlations between GEBV obtained from all 13 scenarios across 10 validation groups were calculated as additional measure to determine the similarities among the scenarios.

RESULTS AND DISCUSSION

Estimated Genetic Parameters

Descriptive statistics for CH_4, BW, and ECM are presented in Table 3. Energy-corrected milk and BW values are consistent with northern European cattle (Manzanilla-Pech et al., 2014; Li et al., 2018). Estimated variances, heritability, repeatability, and phenotypic and genetic correlations for CH_4, BW, and ECM are shown in Table 4. The heritability estimate for CH_4 was 0.14, within the range of estimates from literature (0.10 to 0.30) from smaller studies (van Engelen et al., 2018, Difford et al., 2020). Repeatability for CH_4 was 0.51, meaning that the permanent environmental variation explained a higher proportion of the variance than the genetics for this trait. The estimated heritability for ECM of 0.37 in the current study was in the range of previously reported heritabilities, ranging between 0.27 and 0.54 (Hüttman et al., 2008; Buttchereit et al., 2011; Manzanilla-Pech et al., 2014; Li et al., 2018; Interbull, 2018). Likewise, estimated heritability for BW (0.58) was in the range of estimates in the literature ranging from 0.43 to 0.65 (Berry et al., 2003; Muller et al., 2006; Dechow et al., 2010; Manzanilla-Pech et al., 2014; Li et al., 2018). The genetic correlation between CH_4 emissions and BW (0.50) was close to value of 0.42 reported by Breider et al. (2018) using the SF_6 tracer gas method for measuring CH_4. The genetic correlation between CH_4 and ECM (0.60) in this study was higher than the genetic correlation (0.45) previously reported

Table 3. Descriptive statistics for CH_4, BW, and ECM

Trait	No. of cows	No. of records	Mean	SD	Minimum	Maximum	CV (%)
CH_4[1]	2,230	14,125	572.6	47.1	450.0	699.9	8
BW (kg)	2,714	61,667	641.1	75.2	387.0	899.9	12
ECM (kg)	2,702	61,610	32.9	8.5	10.00	64.8	26

[1]CH_4 is the natural logarithm of ppm, multiplied by 100.

by Lassen and Løvendahl (2016) for CH_4 in grams per day and ECM in Danish Holstein but lower than 0.74 reported by Breider et al. (2018) between CH_4 grams per day and MY in Australian Holstein cows. Also, there is an additional increase in accuracy with multivariate analysis resulting from better connections in the data due to residual covariance between traits (Thompson and Meyer, 1986). These moderate and positive genetic correlations could be used to increase the accuracy of estimation for GEBV of CH_4 when including BW and ECM information on reference animals in the genomic prediction. Furthermore, these genetic correlations will be needed to calculate the correlated response and the genetic gain of these traits in a multitrait index.

Accuracies of GEBV for CH_4

Accuracies of prediction of GEBV for CH_4 averaged across 10 validation groups per method and scenario for BLUP and SSGBLUP are shown in Figure 1.

Per Method. The added information from including genotypes is expected to increase the accuracies; however, this increase was only significant (based on SE) in the base scenario and in sub-scenario 4a. Accuracies of EBV for CH_4 for the base scenario were 0.32 for BLUP and 0.42 for SSGBLUP. For 7 of the 13 scenarios, accuracies of GEBV obtained using SSGBLUP were numerically higher than those obtained using BLUP, as expected based on reports in dairy cattle over numerous traits (Hayes and Goddard, 2008; VanRaden et al., 2009). However, based on the magnitude of standard errors of both methods, these results should be interpreted with caution.

Given the globally insufficient number of animals with CH_4 data available, few studies have reported accuracies of GEBV for CH_4 in cattle. Hayes et al. (2016) reported accuracies between 0.26 and 0.38 for CH_4 traits in Angus beef cattle (CH_4 production rate, methane yield, and 4 definitions of residual methane) using GBLUP and BayesR. Accuracies reported in this current study were higher than those reported by de Haas et al. (2011) for predicted enteric CH_4 emission in Holstein cattle using genomic information (0.37) and pedigree information (0.21).

Across multitrait scenarios, accuracy for BLUP method ranged from 0.10 to 0.72, whereas for SSGBLUP it ranged from 0.12 to 0.75. These results have shown that multitrait prediction performed similarly across methods and on average better than the single-trait scenario. In this study, adding genotype information (SSGBLUP) only significantly increased the accuracy for the single-trait scenario (0.42). This could be partially due to only two-thirds of the animals with predictor trait information having genotypes.

Per Scenario and Sub-Scenario. The higher accuracies of GEBV of CH_4 due to multitrait genomic prediction compared with single-trait genomic prediction are in agreement with previous findings in other traits in Holstein cows, such as linear type traits (Tsuruta et al., 2011), conception rate (Aguilar et al., 2011), and detailed milk protein composition (Gebreyesus et al., 2016). On average, scenarios with CH_4 information performed better than scenarios without CH_4, as in the absence of CH_4 records, the accuracy of estimated GEBV relies entirely on the genetic correlations between CH_4 and routinely recorded predictor traits. This can

Table 4. Estimated genetic, permanent environmental, and residual variances, repeatability, heritability (diagonal), and genetic (below diagonal) and phenotypic (above diagonal) correlations (SE in parentheses) for CH_4, BW, and ECM

	Variance				Correlations		
Trait	Genetic	Permanent environmental	Residual	Repeatability	CH_4	BW	ECM
CH_4	147.6	398.9	500.4	0.51	0.14 (0.05)	0.15 (0.01)	0.25 (0.02)
BW	2,240.7	1,074.8	547.7	0.86	0.50 (0.10)	0.58 (0.03)	0.17 (0.02)
ECM	19.1	15.5	20.4	0.62	0.60 (0.10)	0.26 (0.07)	0.37 (0.03)

be translated as the GEBV is the correlated response of CH_4 when including BW and ECM information. However, the scenarios without CH_4 information in the reference population could result in a phenotype for CH_4 that is only based on ECM and BW, and selecting for this phenotype would likely lead to an unfavorable correlated response reducing ECM and BW if these traits are not included in the selection index.

Furthermore, there are some important messages to point out based on the accuracies obtained in this study across scenarios. First, the scenarios without information on CH_4 but with information on predictor traits in both validation and reference population (3) achieved similar accuracies than the scenarios with CH_4 records and only predictor traits on only the reference population (4). However, the genetic gains achieved via each scenario would vary greatly. In scenario 3 with no CH_4, all the genetic variation of CH_4 comes from ECM and BW, implying that a reduction in CH_4 will in practice be coming from selecting smaller animals that produce less milk, which is counterintuitive to the aim of profitable dairy production. Conversely, scenario 4 with CH_4 information has the full genetic variation of CH_4, including the fraction that is not solely explained by ECM and BW. In principle, in scenario 4 with CH_4 information included, using all traits in a selection index can achieve selection for lower or reduced emitting animals when increasing or maintaining milk production [i.e., improved methane intensity (CH_4/L of ECM)]. Second, scenario 5, which has CH_4 information only in the reference population plus the correlated predictor traits (ECM and BW) in both reference and validation population achieved the highest accuracies. However, because GEBV are usually predicted for young animals before they have their own phenotypes, scenario 5 is not closely aligned with genomic selection schemes in practice, where such a scheme is more related to scenario 4. Yet, this scenario could be important when trying to predict CH_4 in second or later lactation cows (with ECM and BW information available).

Across sub-scenarios, we observed that adding information on BW does not improve the accuracy of prediction of CH_4 compared with adding ECM. This discrepancy in accuracies between predictor traits can be explained by the relatively higher genetic correlation between CH_4 and ECM compared with CH_4 and BW (Table 4). In addition, sub-scenarios with BW and ECM information in both reference and validation populations performed better than the scenarios having the extra information on only the reference population. Similarly, Pszczola et al. (2013) reported higher accuracies of prediction for DMI in Holstein

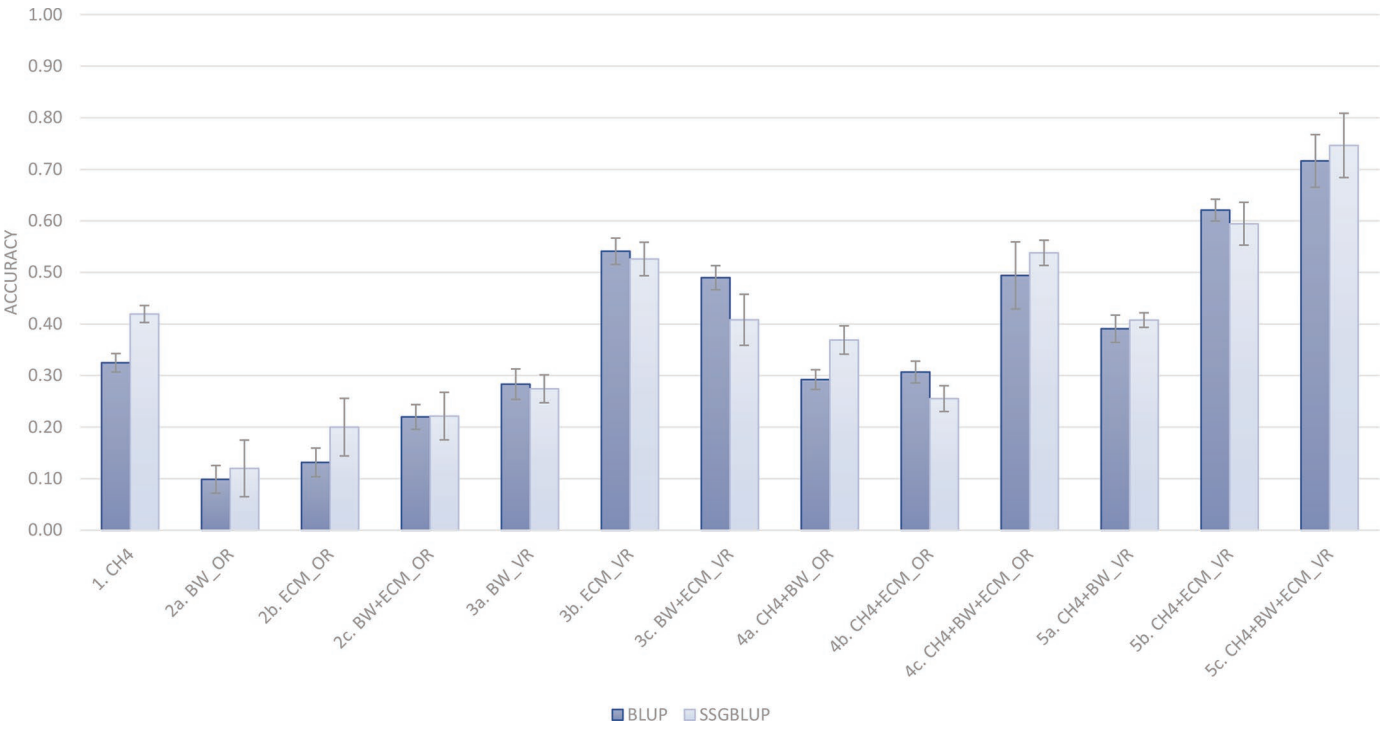

Figure 1. Accuracies of prediction of genomic EBV for methane, averaged across 10 validation groups per sub-scenario for BLUP and single-step genomic BLUP (SSGBLUP). CH_4 = methane concentration, OR = only reference, VR = validation + reference. Error bars represent SE.

cows when including predictor traits in the validation and reference population compared with including them only in the reference population. Additionally, in this study the sub-scenarios including both BW and ECM achieved higher accuracies than the inclusion of only one of them, except for the scenario without CH_4 information but with BW and ECM in both reference and validation population, where a marginal stochastic difference was reported. Likewise, Pszczola et al. (2013) reported higher accuracies (0.62–0.63) when both traits were included compared with including only one trait (0.47–0.57) for BLUP and GBLUP.

As mentioned before, the gain in accuracy of GEBV of the goal trait in multitrait genomic prediction is conditional on the magnitude of the genetic correlations between the goal trait and the predictor traits included in the multitrait analysis. Jia and Jannink (2012) stated that the relative difference in the heritability of the goal trait to predictor trait(s), as in our study (Table 4), also influences the accuracy of the goal trait GEBV, where the gain in accuracy is higher when the heritability of the goal trait is relatively lower than the predictor traits. Within this context, Jia and Jannink (2012) showed the effect of genetic correlation between the traits on the prediction accuracy of the goal trait depends on the magnitude of the heritability estimates of the goal trait, being more remarkable when the heritability of the goal trait is low (0.1) and almost imperceptible when the heritability of the goal trait is high (0.5). This means that for a trait with low heritability it is more important to have higher correlation with the other trait(s) than for a trait with moderate to high heritability. Additionally, Calus and Veerkamp (2011) stated that lowly heritable traits could borrow information from correlated highly heritable traits and consequently achieve higher prediction accuracy.

Bias of GEBV for CH_4

Coefficients of regression are a measure of slope bias in terms of the variance of the GEBV relative to the adjusted phenotype. Figure 2 shows the coefficients of regression of the adjusted phenotype on the GEBV across methods and scenarios. Coefficients larger than 1 indicate underestimation and smaller than 1 indicate overestimation.

Per Method. On average, the regression coefficient for the base scenario (single trait) was not different from the regression coefficients estimated for SSGBLUP, indicating that there is practically no bias on the estimation of the breeding values. For the multitrait scenarios, the regression coefficients varied widely within methods (between 0.34 and 1.02 for BLUP, and 0.48 and 0.95 for SSGBLUP). The regression coefficients did not vary so much across methods for most scenarios.

Per Scenario and Sub-Scenario. In general, the regression coefficients performed consistently per scenario and sub-scenario, similar to the accuracies.

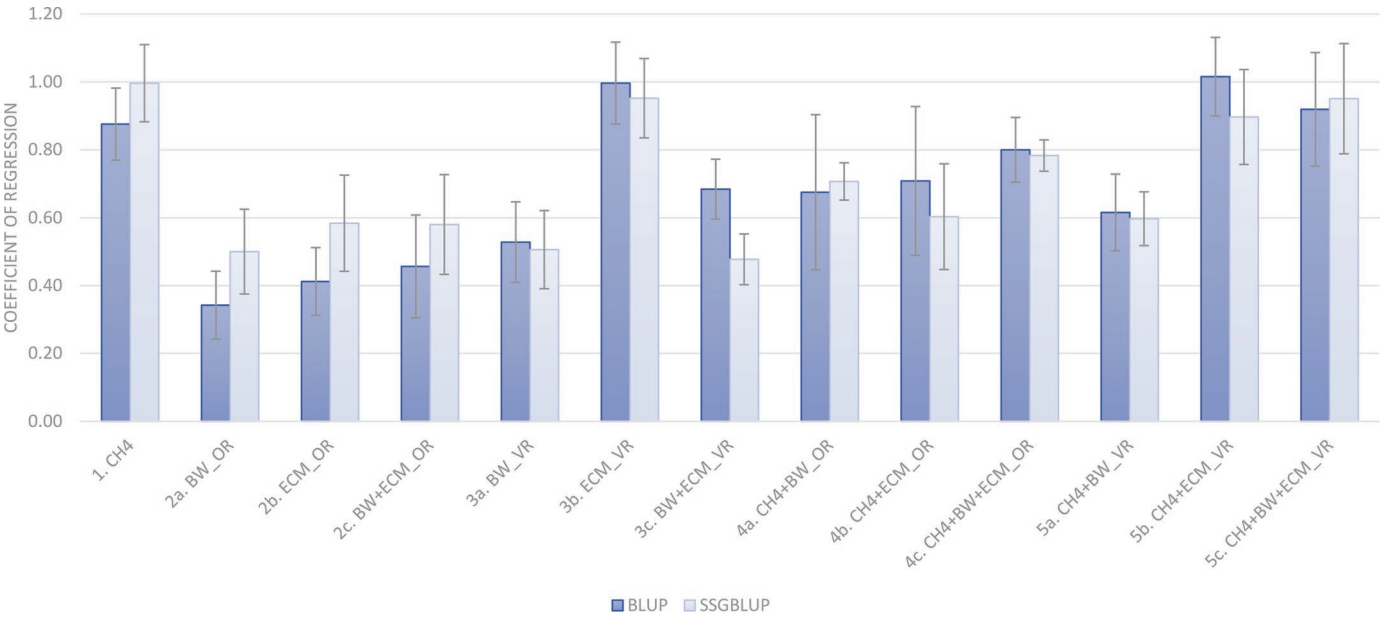

Figure 2. Bias (coefficient of regression) of genomic EBV for methane, averaged across 10 validation groups per sub-scenario for BLUP and single-step genomic BLUP. CH_4 = methane concentration, OR = only reference, VR = validation + reference. Error bars represent SE.

The multitrait scenarios ranked as follows: scenario 2 ranged from 0.34 to 0.64; scenario 3 ranged from 0.48 to 1.11; scenario 4 ranged from 0.60 to 0.88; scenario 5 ranged from 0.60 to 1.06. Sub-scenarios from scenario 3, without CH_4 and with information on BW and ECM only in the reference population, showed more bias than the other scenarios. Additionally, sub-scenarios from scenario 4, without phenotypic observations for BW and ECM in the validation population, showed more bias than scenarios with phenotypic observations for BW and ECM in the validation population as a result of a possible incorrect scale of the variance of the GEBV (Pszczola et al., 2013). Sub-scenarios from scenarios 5, with CH_4 and information on BW and ECM in both validation and reference population, were almost unbiased with regression coefficients closer to unity compared with the other scenarios. In addition, Song et al. (2019) reported that bias reduces in a multitrait genomic prediction (compared with the single trait) when the genetic correlation between the traits is high.

Finally, in terms of bias, our study has also shown that 4 of the 5 sub-scenarios with better accuracies of GEBV also had the least biased regression coefficient estimates, without significant differences from the unity. The 4 sub-scenarios were 3b (ECM_VR; 0.95–1.11), 4c (CH4+BW+ECM_OR; 0.78–0.88), 5b (CH4+ECM_VR; 0.90–1.02), and 5c (CH4+BW+ECM_VR; 0.92–1.06).

Correlations Between Scenarios

In genomic prediction it is common practice to report accuracies and bias of GEBV predictions, but these metrics only reflect the correlation between the predicted GEBV and the true phenotype (in this case the adjusted phenotype) corrected by the heritability of the trait. However, when using multitrait genomic prediction, it is important to understand where the variation of GEBV comes from. One way to do this is calculating the correlation between the GEBV from the different scenarios with the GEBV from the single-trait (base) scenario. In Table 5, we report the average correlations between CH_4 GEBV estimated by SSGBLUP method per scenario across 10 cross-validation groups. The correlations between the GEBV from the multitrait scenarios and the GEBV from the base scenario (single trait) used as a proxy for the best estimate of the true GEBV of CH_4 clearly discriminated between the scenarios which included CH_4 records in the reference population or not. For instance, the correlations between the base scenario and scenarios 2 and 3 were low (0.18–0.33), whereas scenarios 4 and 5, which included CH_4 in the reference population, where moderate to high (0.41–0.81). Furthermore, when comparing multitrait scenarios with and without CH_4 (i.e., scenarios 2 vs. 4 and scenarios 3 vs. 5) the correlations are moderate to high, but not close to the unity. This result demonstrates that including CH_4 information in the reference population is also adding additional information over and above ECM and BW. Scenario 3, which performed similarly to scenario 4 in terms of accuracy and bias, had the poorest correlations with the base scenario GEBV (0.17–0.22). Promisingly, scenario 4, which is the most closely aligned to genomic selection schemes in practice and is the most likely to result in restricted CH_4 with increasing milk production (i.e., dilution of CH_4), had the highest correlations (0.72–0.81) with the base scenario.

Table 5. Average pairwise comparison correlations between genomic EBV estimated by SSGBLUP method per scenario and sub-scenario across 10 cross-validation groups[1]

Scenario/ sub-scenario	1	2 a b c	3 a b c	4 a b c
1				
2				
a	0.18			
b	0.33			
c	0.25			
3				
a	0.17	0.55		
b	0.22	0.53		
c	0.17	0.28		
4				
a	0.79	0.61	0.39	
b	0.81	0.67	0.44	
c	0.72	0.53	0.23	
5				
a	0.67	0.45	0.81	0.73
b	0.68	0.46	0.85	0.72
c	0.41	0.32	0.78	0.70

[1]SSGBLUP = single-step genomic BLUP. OR = only reference; VR = validation + reference. 1 = base scenario. 2a = BW_OR; 2b = ECM_OR; 2c = BW+ECM_OR. 3a = BW_VR; 3b = ECM_VR; 3c = BW+ECM_VR. 4a = CH_4+BW_OR; 4b = CH_4+ECM_OR; 4c = CH_4+BW+ECM_OR. 5a = CH_4+BW_VR; 5b = CH_4+ECM_VR; 5c = CH_4+BW+ECM_VR.

Implications

In terms of gain in accuracy (in percentage) compared with the base scenario, our results have shown that including information on ECM and BW can increase the accuracy of GEBV for CH_4 from 29% (scenario 4) up to 90% (scenario 5). However, the most feasible multitrait scenario, not only in terms of increased accuracy compared with the single-trait scenario but also one that could be used in practice when predicting CH_4 for young candidates, is the scenario with CH_4 and information

on ECM and BW only in the reference population. Furthermore, we should be aware that part of the gain in accuracy when using ECM to predict CH_4 is partially due to the higher accuracy of predictions for ECM, which were 0.50 and 0.65 for the base scenario (BLUP and SSGBLUP, respectively), much higher than the estimates for CH_4. Moreover, the increase in accuracy using predictor traits in a multitrait approach has a plateau, meaning that there is a maximum of improvement that can be achieved by adding information on correlated traits. In addition, although using only ECM and BW records to predict CH_4 in the absence of CH_4 records leads to increased individual accuracies in CH_4, this scenario can lead to a reduction of methane only at the cost of genetic gain for MY. Therefore, recording CH_4 in more animals remains a priority. To restrict or reduce gain in CH_4 without reducing gains in ECM or BW, a multitrait selection index is needed where all the economically important traits are included. A recent study (C. I. V. Manzanilla-Pech, unpublished data) shows that is possible to reduce methane and still have a positive genetic gain on ECM using 2 strategies, either applying a penalization (negative economic value for CH_4) or by including a negative economic value for residual feed intake. Both strategies would lead to a reduction on CH_4 without compromising the genetic gain on ECM and will represent an improvement in terms of CH_4 per liter of milk.

Finally, is it well known that heritability is a determinant factor for the calculation of the optimal reference population size, and this is directly proportional to the maximum accuracy achieved in the genomic prediction (Daetwyler et al., 2010). Given that in the CH_4 case both are limited (low heritability and small reference population size), it is recommended to investigate the optimal reference population size (and structure) that maximize the accuracy of prediction of GEBV for CH_4 with the current heritability. Finally, there are other important traits highly correlated with CH_4 that could contribute to increase the accuracy of prediction of GEBV for CH_4, such as feed intake, feed efficiency (residual feed intake), and energy balance, among others. However, given that these are scarcely recorded traits, collecting information on them and further investigating on their interactions would be needed.

CONCLUSIONS

Multitrait genomic prediction leads to higher prediction accuracy than traditional single-trait genomic prediction, particularly when predictor traits are highly genetically correlated with the goal trait. This is beneficial for scarcely recorded traits, where phenotypes are not available on all individuals but routinely recorded traits are. Adding genotypic information to increase the accuracy of prediction for CH_4 showed a significant advantage for the base single-trait scenario but not in the multitrait scenario. We conclude that the most feasible multitrait scenario in terms of feasibility when predicting CH_4 for young candidates is the scenario with CH_4, ECM, and BW information in the reference population. This scenario also proved to be the one most correlated with the base scenario.

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