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# Implementation of Methane Efficiency Evaluations for Canadian Holsteins

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## Abstract

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

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

## Introduction

Concerns about the effects of climate change on environmental sustainability are growing. Numerous global dairy industry stakeholders, including Dairy Farmers of Canada, have made commitments to achieve net-zero greenhouse gas (**GHG**) emissions by 2050. Methane (**$CH_4$**), a potent GHG, which remains in the atmosphere for about 12 years and makes up 14% of Canada's GHG emissions, has been under the spotlight as it is responsible for nearly half the net global temperature change due to human activities in the last decade (Environment and Climate Change Canada, 2022).

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

## Materials and Methods

### *Data*

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

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

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

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

### *Model*

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

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

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

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

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

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

### *Derivation of Methane Efficiency*

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

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

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

with

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

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

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

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

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

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

$$Y_1 = \text{fixed}_1 + \text{random}_1 + e_1$$
$$Y_2 = \text{fixed}_2 + \text{random}_2 + e_2$$
$$Y_3 = \text{fixed}_3 + \text{random}_3 + e_3$$
$$Y_4 = L_{41}*Y_1 + L_{42}*Y_2 + L_{43}*Y_3 + \text{fixed}_4 + \text{random}_4 + e_4,$$

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

### *Genomic Evaluation*

A four-trait Single-Step genomic evaluation was implemented at Lactanet Canada using MiX99 and related software (MiX99 Development Team, 2017). The April 2023 data included 134 963 genotyped animals, with 68 138 genotyped cows with records and 7 921 genotyped sires. Animals were genotyped either with 50K SNP panel or a low-density panel and imputed to 50K using FImpute (Sargolzaei al., 2014). The genomic relationship matrix (**G**) is constructed by VanRaden Method I. (VanRaden, 2008), and **G** is blended with the additive relationship matrix (**A**) assuming that 80% of the total genetic variance was explained by SNP effects. Scaling of **G** and **A** is performed using the Christensen (2014) method. The APY algorithm for Proven and Young (Misztal et al., 2014) is applied for inversion of **G**, with the core population of 25 000 (the oldest genotyped animals in the Lactanet database). Groups for unknown parents are not included in the model. The SNP effects, to be used for calculating Genomic Estimated Breeding Values (**GEBV**) for genotyped animals not included in the single-step core analysis, are estimated from the GEBV of reference animals (as in Lourenco et al., 2015).

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

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

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

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

### *Relative Breeding Values*

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

### *Genetic Correlation Between Collected and MIR-Predicted Methane*

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

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

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

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

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

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

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

## Results and Discussion

### *Genetic Parameters*

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

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

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

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

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

[1] Approximated SE <0.03

### *Genomic Evaluations*

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

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

### *Relationship with Collected and Predicted Methane Emissions*

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

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

### *Expression and Expected Response*

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

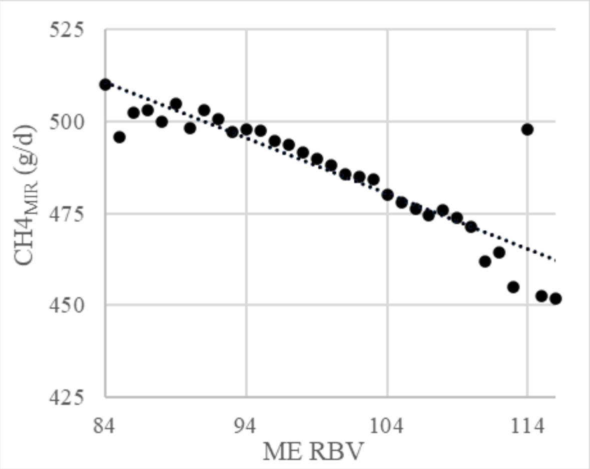

**Figure 1:** Daughter average $CH4_{MIR}$ averaged by sire RBV for ME

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

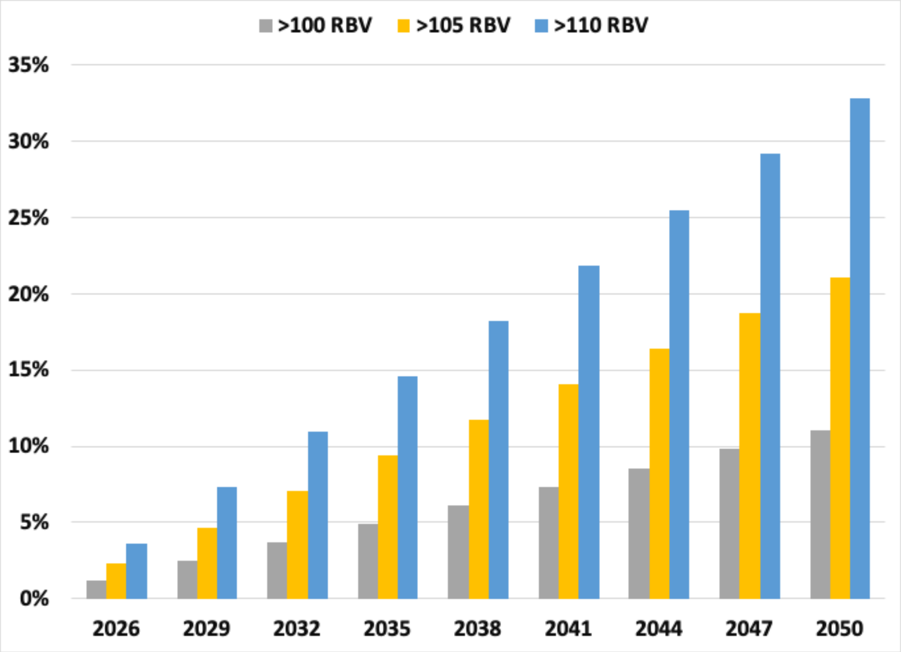

**Figure 2**: Expected selection response for three different scenarios: a) grey if top 50% bulls are selected; b) yellow if bulls over 1 SD for ME are selected; and c) blue if bulls over 2 SD are selected

## Conclusions

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

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