

Assessment of methane emission traits in Canadian Holstein cows

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ABSTRACT

ASSESSMENT OF METHANE EMISSION TRAITS IN CANADIAN HOLSTEIN COWS

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Enteric methane production from ruminants represents a loss of productive energy and contributes to greenhouse gas emissions. This thesis presents a phenotypic data description and genetic parameter estimates of three methane emission traits (methane production, yield, and intensity) in Canadian Holstein cows. The variation of methane production across different times of recording within a day was also assessed. Lastly, the potential re-ranking of estimated breeding values for the alternative methane traits was evaluated. Methane emission traits were found to be moderately heritable, and moderate to high genetic correlations between pairs of methane traits were found. Therefore, genetic selection for methane emission is possible. Direct selection for one trait will indirectly improve the other traits, however, substantial re-ranking of estimated breeding values was observed, especially among the sires. Thus, the methane emission trait of choice will depend on how it will possibly be included in the genetic evaluation program.

Keywords: Holstein cows, methane traits, selection

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TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF SYMBOLS AND ABBREVIATIONS	viii
CHAPTER 1: INTRODUCTION AND OBJECTIVES	1
1.1 GENERAL INTRODUCTION.....	1
1.2 OBJECTIVES.....	3
1.3 LITERATURE CITED	4
CHAPTER 2: LITERATURE REVIEW	8
2.1 INTRODUCTION	8
2.2 HOW METHANE IS PRODUCED IN THE RUMEN	9
2.3 METHODS FOR MEASURING METHANE EMISSIONS FROM RUMINANTS ...	11
2.4 METHANE EMISSIONS AS A BREEDING GOAL.....	15
2.5 CHALLENGES OF INCORPORATING METHANE EMISSIONS INTO BREEDING SCHEMES	18
2.6 STANDARDIZATION OF NATIONAL METHANE EMISSION DATA SETS	20
2.7 FINAL REMARKS.....	21
2.8 LITERATURE CITED	23
2.9 FIGURES	35
CHAPTER 3: ASSESSMENT OF METHANE EMISSION TRAITS IN CANADIAN HOLSTEIN COWS	37
3.1 ABSTRACT.....	37
3.2 INTRODUCTION	39
3.3 MATERIALS AND METHODS	41
3.3.1 <i>Data Collection</i>	41
3.3.2 <i>Variation in methane testing</i>	43
3.3.3 <i>Data set and Methane Traits</i>	44
3.3.4 <i>Statistical analysis and Connectedness</i>	45
3.3.5 <i>Variance components</i>	46
3.3.6 <i>Rank correlations and accuracy of estimated breeding values</i>	47
3.4 RESULTS AND DISCUSSION	48
3.4.1 <i>Descriptive Statistics</i>	48
3.4.2 <i>Variation over Time</i>	49
3.4.3 <i>Genetic parameter estimates</i>	50
3.4.4 <i>Correlations among methane traits</i>	53
3.4.5 <i>Accuracy of EBVs and EBV Rank Correlations</i>	54
3.5 CONCLUSIONS.....	55

3.6	LITERATURE CITED	57
3.7	TABLES	67
3.8	FIGURES	71
CHAPTER 4: GENERAL DISCUSSION		73
4.1	FINAL REMARKS.....	73
4.2	LIMITATIONS AND FUTURE RESEARCH STEPS	75
4.3	LITERATURE CITED	77

LIST OF TABLES

Table 3.1. Number of Holstein cows and records, mean, standard deviation (SD), minimum, maximum, and coefficient of variation (CV) of methane production (MeP), methane yield (MeY), and methane intensity (MeI).	67
Table 3.2. Estimated additive genetic (σ_{2a}) permanent environmental (σ_{2pe}) residual (σ_{2e}) variances, heritabilities (h^2), repeatability (r), and average accuracy of EBVs for methane production (MeP), methane yield (MeY), and methane intensity (MeI).	68
Table 3.3. Estimated additive genetic (above) and phenotypic (below) correlations with their corresponding (SE) between pairs of methane traits, i.e. methane production (MeP), methane yield (MeY), and methane intensity (MeI).	69
Table 3.4. Estimated EBV rank correlation for sires with daughters (above diagonal) and for all animals (below diagonal) between pair of methane traits, i.e. methane production (MeP), methane yield (MeY), and methane intensity (MeI).	70

LIST OF FIGURES

Figure 2.1. Fermentation process in the reticulorumen and hindgut. Sugars are fermented to VFA through multi-step pathways producing reduced equivalents	35
Figure 2.2. Layout of the GreenFeed (C-Lock Inc., Rapid City, SD) system used at University of Guelph (the flux method). RFID = radiofrequency identification (Hristov et al. 2015).	36
Figure 3.1. Daily methane production from dairy cows fed once daily, measured using GreenFeed. Means between T3 and T2 were not significantly different and variances between T1 and T3, as well as T3 and T4, were not significantly different ($P > 0.05$).	71
Figure 3.2. Heritability estimates for a single record and for the average of different numbers of methane emission records (n= from 2 to 5) for methane production (MeP), methane yield (MeY), and methane intensity (MeI).	72

LIST OF SYMBOLS AND ABBREVIATIONS

AHCS: Automated Head-Chamber System

AIREML: Average Information Restricted Maximum Likelihood

AMS: Automated milking system

BW: Body weight

CG: Contemporary Group

CH₄: Methane

CO₂: Carbon dioxide

CV: Coefficient of Variation

DMI: Dry matter intake

DMP: Daily Methane Production

DRTC: Dairy Research and Technology Centre

EBV: Estimated breeding value

EDGP: Efficient Genome Dairy Project

FA: Fatty acids

FE: Feed efficiency

GHG: Greenhouse gas

H₂: Hydrogen

h₂: Heritability

LPI: Lifetime Profit Index

MIR: Mid-infrared

MY: Milk yield

MeI: Methane intensity

MeP: Methane production

MeY: Methane yield

ODRC: Ontario Dairy Research Centre

RFI: Residual feed intake

RFID: Radio Frequency Identification

RPM: Residual methane production

SE: Standard Error

SF₆: sulfur-hexafluoride

TMR: Total Mixed Ration

VFAs: Volatile fatty acids

CHAPTER 1: INTRODUCTION AND OBJECTIVES

1.1 GENERAL INTRODUCTION

Increased dairy production is necessary to fulfill the future demands for animal proteins, as the Food and Agriculture Organization of the United Nations has projected the global population to reach 9.73 billion people by 2050 (FAO, 2017). In particular, economic growth and accelerated urbanization contribute to significant changes in population-level dietary habits in low- and middle-income countries, meaning that much of the increase in the global demand for food of animal origin is seen in developing countries (Henchion et al. 2017). However, with constant pressure to increase production, two important challenges need to be addressed, efficiency and environmental sustainability. Enteric fermentation in ruminant animals is a natural part of the digestive process in which microbes in the digestive tract, or rumen, decompose and ferment food, producing methane (CH₄) as a by-product. Enteric fermentation represents 17% of the global Methane sources (Knapp et al. 2014) and 6-11% of feed energy losses in ruminants (Appuhamy et al., 2016).

Canada is one of the most industrialized countries and in its emissions profile carbon dioxide is the largest contributor to greenhouse gas (GHG) emissions at 80%, followed by methane emissions (13%) and nitrous oxide (5%) (National Inventory Report, 2019). In 2018, 8.1% of Canada's emissions were associated with the agricultural industry, from which livestock digestion (enteric fermentation) accounted for 41% (National Inventory Report, 2020). Thus, retailers and consumers are increasingly concerned about the impact of GHG emissions on the environmental footprint of foods. In order to address these concerns, Canada has pledged to reduce its total GHG emissions by 30% by 2030 after signing the Paris Agreement in 2016. One way to help achieve

these goals is by increasing the sustainability of the food production system, of which the dairy industry is a major part of.

In recent years, research focusing on the genetic background of methane emission traits has become of interest in many dairy-producing countries, such as the Netherlands, Australia, and Spain (de Haas et al. 2011; Moate et al. 2016; González-Recio et al. 2020). However, with this interest comes the challenge of collecting enough methane emission phenotypes, especially because the recording is done primarily in research station facilities as opposed to commercial farms due to the specialized equipment and resources necessary. Therefore, through projects, such as The Efficient Dairy Genome Project (<https://genomedairy.ualberta.ca/>), an international collaboration to develop a larger reference population will allow for the use of genomic technologies to develop a methane emission trait evaluation.

Prior to defining a phenotypic trait to include in genetic selection indices it is required to have a precise understanding of breeding goals. Selection goals around methane emissions involve identifying animals with the same production yield, but with less enteric methane produced or have higher production yield, but with the same level of enteric methane production. There are many ways to express methane emission, such as methane production- defined as liters or grams per day; methane intensity- defined as liters or grams of methane related to output (e.g., kg of milk or kg of meat); or at methane yield- defined as liters of methane associated to input (e.g., per kg of DMI) (Herd et al. 2013; de Haas et al. 2017; Brito et al. 2018). Residual methane production, defined as observed minus predicted methane production, has also been proposed (Herd et al. 2014; Berry et al. 2015; Manzanilla-Pech et al. 2016). Consequently, to define a phenotypic methane emissions trait for genetic selection, knowledge of its heritability, and interactions with other traits of economic importance is essential. As reviewed by Brito et al. (2018), international estimates

suggest that most methane traits are moderately heritable. Furthermore, genetic correlations between methane traits and other economically important traits have been estimated in different populations (e.g., Pickering et al. 2015; Kandel et al. 2017; Breider et al. 2019), which have shown methane production to have unfavorable correlations with milk production and feed intake. Therefore, although there is interest in including methane emission in the breeding goal, careful consideration should be given to alternative methane traits that adjust for production or feed intake.

1.2 OBJECTIVES

The goal of this study was to investigate potential methane traits to be included in future breeding goals of the Canadian dairy industry. Within this goal, the two specific objectives were:

1. To determine which methane trait should be included in future selection indices.
Specifically investigating the traits: methane production, methane yield, methane intensity.
2. Estimate genetic parameters, as well as genetic correlations among methane traits.

This thesis is divided into three research chapters. Chapter 2 provides an overview of enteric methane emissions from cattle, the different methodologies for measuring methane emissions, a review on genetic parameters of methane emission traits and their genetic correlations with production traits, challenges of incorporating methane emissions traits in breeding schemes and the standardization of methane emission data necessary for international collaboration. Chapter 3 presents the first heritability estimates for methane emission traits in Canadian Holstein cattle, including methane production, methane yield, and methane intensity, in addition to estimated genetic correlations among these traits. Lastly, Chapter 4 summarizes the significance of the results to the Canadian dairy industry, what traits may be the best for future selection schemes, and the future of establishing a reference population for methane emission traits.

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CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Greenhouse gas (**GHG**) emissions have been a primary driving force behind increased temperatures worldwide, contributing to climate change (Gillet et al. 2012). The effect of greenhouse gases is likely due to the high absorption of solar infrared (IR) radiation by gases and the earth's surface, which are heated and remitted as IR radiation at a low frequency (Moss et al. 2000). The most prevalent naturally occurring greenhouse gases in order of most abundant to least are water vapor, carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and ozone (O₃). Although these greenhouse gases are essential for maintaining life on earth, it has been accepted that their rising concentrations should be halted. Consequences involving the increase in average global temperatures impacting both human life and animals are resulting from elevated greenhouse gases responsible for the greenhouse effect. These consequences include the availability of water supplies, a new distribution of wet areas and deserts around the globe, and alteration of the range/number of pests that affect plants or diseases that threaten animal or human health (Moss et al. 2000).

Although carbon dioxide (CO₂) receives the most attention as a global warming factor, mitigation of enteric methane emissions has become an increasingly critical area of research because methane also plays a vital role in global warming. Enteric methane is one by-product of the digestive process in ruminants and is expelled by the animal through eructation. Methane (CH₄) has 28 times the global warming potential of CO₂ as it has a much higher heat-trapping

ability. Therefore, the reduction of CH₄ would decrease the impact of greenhouse emissions (Smith et al. 2007; Myhre et al. 2013). The Food and Agriculture Organization of the United Nations (FAO, 2013) stated that livestock is responsible for 14.5% of global GHG emissions, with ruminants contributing 80% of those emissions, making the agricultural industry a noteworthy contributor for GHG emissions (Gerber et al. 2013). The ruminant population is responsible for an estimated 1.8 billion metric tons of CO₂-equivalents per year of enteric fermentation, making it the most significant contributor to the increase in global greenhouse gases among the agricultural sector (O'mara, 2011; Herrero et al. 2011). Enteric CH₄ fermentation by ruminant's accounts for about 40% of total non-CO₂ emissions from agriculture. Following beef cattle (45%), dairy cows were the second-highest methane source at 26% (FAO, 2017) among ruminants. As a colorless and odorless gas, enteric methane in cattle is produced in the rumen by anaerobic *archaeal* microorganisms. They convert hydrogen (H₂) and CO₂ to CH₄, which is predominantly emitted via eructation from the lungs and is the primary source of total CH₄ produced by ruminants (de Haas et al. 2017). Methane production represents an energy loss for ruminants, dependent on diet, ranging from 3 to 12% of the gross energy intake being lost as enteric methane (Johnson and Johnson, 1995). Therefore, the mitigation of CH₄ emissions from livestock has two advantages: a decrease in its environmental impact and an increase in energy efficiency.

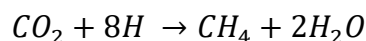
2.2 HOW METHANE IS PRODUCED IN THE RUMEN

Microbes in the ruminant have a symbiotic relationship with the host, where both benefit from converting complex plant carbohydrates to energy. The microbes in the ruminant consist of bacteria, protozoa, fungi, and archaea (Knapp et al. 2014). As an anaerobic environment, the rumen ecosystem functions differently from other anaerobic ecosystems (e.g., wetlands and estuaries),

where degradation of plant material occurs in a shorter time frame and products yielded from fermentation are different (Knapp et al. 2014). As a naturally occurring process in ruminants, CH₄ is a by-product of anaerobic respiration with a predominant portion (~90%) produced in the rumen and a small portion (~10%) in the hindgut (Ellis et al. 2008). Methanogens of ruminants belonging to the archaea domain have been identified to consist of three major genera (*Methanobrevibacter*, *Methanomicrobium*, and *Methanosphaera*) and three minor genera (*Methanosarcina*, *Methanobacterium*, and a large group of uncultured rumen archea labelled as *Thermoplasmatales* also known as rumen cluster C) through population size and functionality (Janssen and Kirs, 2008; Kong et al. 2013; Poulsen et al. 2013; Patra et al. 2017). Methanogens are found in both the rumen and hindgut, however they differ between these two compartments regarding population structure, microbial metabolism, and ecology.

Hydrolyzation of carbohydrates in the reticulorumen and hindgut occur via enzymatic breakdown of feed sources and fermentation by microbes in the rumen. The fermentation process requires enzymatic activity to produce volatile fatty acids (VFAs), and it is accomplished through multi-step pathways that produce metabolic hydrogen (Figure 1) (Hungate, 1966; Czerkawski, 1986; Moss et al. 2000).

Metabolic hydrogen is converted to H₂ by bacterial species that express hydrogenase, the H₂ is then converted into CH₄ in the combined reaction known as Methanogenesis:



Methanogenesis is a vital part of energy metabolism in ruminants. Thus, it is crucial to measure its production in understanding ruminant livestock productivity. Functions of the rumen

microbiome (e.g., metabolic and digestion) can be combined with CH₄ emissions data to provide further information on the intricacy of efficient livestock systems (Firkins and Yu, 2015). Therefore, by understanding how enteric methane is produced and the outcome of microbial products produced during fermentation processes, further research can be conducted on how to manipulate productivity in livestock. The role of methanogens is to prevent ruminal fermentation pathways to be inhibited through negative feedback mechanisms by removing dissolved H₂. If the H₂ is not dissolved, this results in the reduction of the overall breakdown of carbohydrates, the rate of microbial growth, and of microbial protein synthesis. (McAllister and Newbold, 2008). Mitsumori et al. (2012) found that increasing metabolic H₂ into VFAs or changes in biochemical processes that result in alternative biochemical pathways that are thermodynamically favorable, can achieve a reduction in CH₄ production. For example, increased lipid content in feed by unsaturated fatty acids is thought to decrease methanogenesis. Unsaturated fatty acids can be used as an alternative to carbon dioxide reduction as hydrogen-acceptors. Additionally, recent research identified that there are methanogens that use methyl donor compounds and CO₂ instead of H₂, indicating that there are possible pathways that still need to be discovered that generate CH₄ (Poulsen et al. 2013). Therefore, the relationship between CH₄ production and intake of digestible organic matter by the animal is nonlinear, adding to the complexity and difficulty of predicting CH₄ emissions and developing mitigation strategies.

2.3 METHODS FOR MEASURING METHANE EMISSIONS FROM RUMINANTS

Several *in vivo* measurement techniques have been applied in livestock to quantify methane emissions, such as the respiration calorimetry (either closed or open-circuit), polyethylene tunnel system, isotope dilution, tracer gas [i.e., sulfur hexafluoride (SF₆)], Automated Head-Chamber

System (**AHCS**, GreenFeed®), and micrometeorological mass balance (Kebreab et al. 2006). With growing research, newer technologies are being developed to assess CH₄ concentrations, using rumen sensors to study on an hourly basis (Laporte-Urbe and Gibbs, 2009) or by measuring CH₄ in commercial settings via eructation during eating at pasture feeders or in robotic milking stations (Utsumi et al. 2011). Comparisons of the measurement techniques have identified systematic differences (Kebreab et al. 2006; Muñoz et al. 2012). In research settings, respiration chambers remain the optimal strategy that permits animal confinement and allows for the development of predictive models for estimating greenhouse gas inventories of ruminant livestock emissions (Knapp et al. 2014). However, their use can be tedious and time-consuming and present a different environment to pastoral grazing. Also, despite producing favorable results due to its accuracy and low coefficient of variation, other variables are restricted using this method. These include diet selection, and the rate and frequency of eating that may affect individual animal emissions in the production environment (Jonker et al. 2014; Pacheco et al. 2014). Therefore, enhancement of the other existing methodologies or development of new methods that allow for repeatable, accurate methane emission measurements from different housing situations or groups of animals is crucial in evaluating mitigation strategies.

The sniffer method is a recently emerged method that is non-invasive, high-yielding, and feasible in commercial settings (Lassen et al. 2012, Garnsworthy et al. 2012a). During individual milking, the air is consistently examined from the concentrate container of automated milking systems (**AMS**), where test gas concentrations are recorded. Two conventional techniques are the Gasmeter DX-4000 (Gasmeter; Gasmeter Technologies Oy, Helsinki, Finland) (Lassen et al. 2012, Haque et al. 2015) and the Guardian NG/Gascard (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK) (Garnsworthy et al. 2012a, Garnsworthy et al. 2012b, Bell et al. 2014a, Bell et

al. 2014b). Although these systems differ in calculations and techniques, the Gasmeter DX-4000 generates a prediction equation based on the ratio of CH_4/CO_2 and two gas concentrations (Madsen et al. 2010). While the Guardian NG/Gascard involves the rate of methane emissions and a scaling factor (Garnsworthy et al. 2012a), both systems require readings of gas concentration levels for their outputs. However, constraining the animal to the device's interface poses to be an implication associated with the non-invasiveness of these techniques, which allows for erroneousness and inaccuracy amongst readings caused by air commotion with the AMS and the cow's head movement in the AMS concentrate container (Huhtanen et al. 2015). To correct this and reduce the sources of error to obtain a phenotype (e.g., average CH_4/week), continuous repeated spot samples for a few days was required for appropriate ranking of animals (Hegarty, 2013; Hill et al. 2016).

In addition, there is another method called the sulfur hexafluoride (SF_6) tracer gas technique that is widely used, which utilizes the "flux" method (Johnson et al. 1994). Analysis of gas samples for SF_6 and CH_4 were previously described by Deighton et al. (2014). Briefly, this method was developed in an attempt to eliminate the confining factor that is associated with respiration chambers. In the vicinity of the animal's nostrils, air is collected through a tube attached to a halter and connected to an evacuated canister worn around the neck or back. A capillary tube or orifice plate is used to limit airflow through the tube so that in approximately 24 hours the canister is filled up to 50-70 percent. The animal's rumen is equipped with a permeation tube containing SF_6 with a pre-determined release rate of SF_6 that is then multiplied by the ratio concentrations of CH_4 to SF_6 in the canister to measure the CH_4 emission rate. Despite being correlated with respiration chambers, the limitations associated with the SF_6 method include

variability between-cow measurements, is labour intensive, and highly dependent on the release rate of SF₆ (Grainger et al. 2007; Muñoz et al. 2012; Huhtanen et al. 2015).

A newly developed system, called the GreenFeed™ (C-lock Inc. 2015), also utilizes the “flux” method. This device provides similar daily methane production rate values as the respiration chamber and SF₆ technique, but shows lower variability for methane emissions compared to the SF₆ method (Hammond et al. 2016; Hristov et al. 2016). The GreenFeed machine uses the same concept for measuring gases in respiration chambers, where emitted air is captured via an induced active airflow (Figure 2). This method involves air flow, integration of gas concentrations measurements, and recognition of muzzle position to allow precise measurement of CH₄ and CO₂ fluxes to be recorded throughout each animal trip to the feed trough (Huhtanen et al. 2015). More specifically, it combines automatic dispensing of feed (e.g., pellets) with measurements of CO₂ and CH₄. Animals that enter an automated feeding system are detected, and CH₄ and CO₂ concentrations are assessed. To quantify the flow and, thus, CH₄ and CO₂ emitted while eating, the air is continuously pumped through the automated feeding system. Additionally, the system can automatically execute recovery experiments by issuing small amounts of tracer gas inside the feeder’s trough to determine how much of the expiration air is collected. Ideal applications for this device would be in conventional tie-stalls, inside AMSs, and grazing animals-fed supplements. Like the sniffer method, the same disadvantage is encountered as it relies upon having the animal’s head within the feed trough. Therefore, correlations with whole-day emissions need to be thoroughly examined (Storm et al. 2012). However, when compared to the SF₆ technique the GreenFeed machine, has shown lower variability for methane emissions

Huhtanen et al. (2015) compared the sniffer method to the flux method. They found that CH₄ and CO₂ concentrations determined by the sniffer method were inadequately correlated with

respective gas fluxes, and only loosely based relationships between gas concentrations and other biological parameters (e.g., dry matter intake (**DMI**) or body weight (**BW**) were observed. It was suggested that the sniffer method's gas concentrations reflect its high dependency on muzzle proximity to the sample intake. Thus, factors such as muzzle movement and variable air-mixing conditions within the feed trough could explain the higher variations observed in enteric CH₄ emissions between cows. Therefore, improving the sniffer method's accuracy may not be possible by increasing the number of replicates as the cow muzzle position behaviour is highly repeatable. However, in the flux method, these variables are accounted for by an induced airflow system, which helps control the mixing and muzzle-position sensor to avoid inaccurate measurements of CH₄ emissions. Therefore, under commercial conditions, the flux method seems to be the superior method for measuring CH₄ emissions.

2.4 METHANE EMISSIONS AS A BREEDING GOAL

A precise understanding of breeding goals is required before defining a phenotypic trait is used for genetic selection. Selection goals around methane emissions involve to identify animals that have the same production yield, but with less enteric methane produced or have higher production yield, but with the same level of enteric methane production. In addition to define a phenotypic methane emission trait for genetic selection, knowledge of its heritability and interactions with other traits of economic importance is essential.

Various systems have been developed to optimize direct measurement of CH₄ emissions or to predict CH₄ emissions for an individual animal, including feeding behavior and feed intake, rumen function, metabolites and microbiome (rumen microbiota and host-microbiome interactions), milk production and composition, hindgut and feces, and measurements at the level

of the whole animal (de Haas et al. 2017; Negussie et al. 2017). In addition, alternative expressions of direct measured CH₄ emissions have been proposed (Herd et al. 2013; Brito et al. 2018). Some examples are:

i) CH₄ production (MeP): defined as methane production rate per time unit e.g. grams per day, kg per day, or liters per day;

ii) CH₄ intensity (MeI): defined as methane production rate per unit of product e.g. g of CH₄ per kg/L of milk produced a day or kg of carcass;

iii) CH₄ yield (MeY): defined as methane production rate per kg of DMI e.g. grams per kg of DMI;

iv) Residual CH₄ production (RPM): observed methane production minus predicted methane production.

Heritability (**h₂**) estimates for methane traits in Holstein dairy cattle have recently begun to emerge. Lassen and Løvendahl (2016) reported h₂ estimates of 0.21 (\pm 0.06) for both MeP (in grams/day) and MeI (grams/liter of milk produced). Similarly, Pszczola et al. (2017) found h₂ of 0.21 (\pm 0.12) at the beginning of lactation for MeP measured in grams/day. In addition, some authors estimated h₂ between 0.12 (SE 0.005-0.06) and 0.44 (\pm 0.016-0.10) for MeP predicted from milk fat composition (Kandel et al. 2015; van Engelen et al. 2015; Vanrobays et al. 2016). Manzanilla-Pech et al. (2016) evaluated several CH₄ traits in Angus beef cattle. They validated them in a lactating Holstein population reporting h₂ estimates of 0.23 for MeP, 0.30 for MeY, and 0.42 for MeI, all with high SE (approximately 0.23). Whereas, van Engelen et al. (2018) investigated h₂ for predicted methane traits based on milk mid-infrared spectra reported estimates of 0.17 for MeP, 0.21 for CH₄ yield, and 0.18 for MeI with SE of 0.07. These studies indicate that CH₄ emission traits in dairy cattle are moderately heritable, and thus, genetic selection is possible.

Previous studies investigating the genetic correlations between methane emissions and production traits suggest that selection based on production will lead to an increase in methane emissions (Kandel et al. 2015; Lassen and Løvendahl 2016; Breider et al. 2019; Pszcola et al. 2019). Kandel et al. (2015) estimated genetic correlations between mid-infrared (**MIR**) predicted CH₄ traits and milk yield (**MY**) and found low and negative correlations (-0.11 to -0.18) in early lactation, but positive correlation with milk composition traits such as fat content (0.31 to 0.55) and protein content (0.14 to 0.36). Similarly, Pszcola et al. (2019) reported positive correlations between MeP with protein content (0.07) and fat content (0.21). However, their findings showed a positive correlation with milk production (0.15) along with Breider et al. (2019) reporting a range of correlations from 0.38 to 0.57. These differences in correlations between CH₄ and milk production can be explained by the lactation period of the cow when the methane was measured, as Pszcola et al. (2019) and Breider et al. (2019) measured methane emissions in late lactation. These results are to be expected, as in early lactation, since cows enter a negative energy balance, and substantial body energy is utilized in addition to energy from DMI for milk production. Therefore, the correlation between MY and MeP can be negative at the beginning of lactation because, during this stage, lower amounts of CH₄ is being produced due to the cow utilizing her body energy reserves for milk production (Breider et al. 2019). Whereas, in later lactation, energy for milk production originates from DMI, resulting in methane production (Grainger et al. 2007; Breider et al. 2019). This explains the positive correlations observed between MY and CH₄ production in later stages of the lactation. Also, positive correlations (0.30 to 0.47) have been found between fatty acids (**FA**) profiles and predicted MeP (Dijkstra et al. 2011; Mohammed et al. 2011). Feed digestion in the rumen results in the by-products methane and VFA (Ellis et al. 2008). Specifically, VFA and FA can then be absorbed into the blood from the feed and used in

the mammary gland's milk fat production (Hristov et al. 2015). This implies that selecting for increased milk production with greater fat content can result in a rise in methane production expressed in the amount of gas per cow.

Additionally, traits related to methane also include BW and feed efficiency (**FE**) traits, such as residual feed intake (**RFI**) (Hegarty et al. 2007). RFI is defined as the difference between actual and predicted DMI, which is the DMI corrected for energy requirements, including maintenance, lactation, and pregnancy (Kennedy et al. 1993). It has been reported that beef cattle with low RFI, in addition to be more feed efficient, they produce lower emissions (Basarab et al. 2013) than cattle with high RFI. A genetic correlation equal to 0.44 was found between RFI and CH₄ emissions in beef cattle (de Haas et al. 2011), suggesting that selection on RFI may be one strategy to reduce CH₄ emissions from cattle. However, when determining which phenotype to use for genetically selecting for FE, it is important to note that selecting only for a lower DMI there is a potential risk of negatively impacting traits of economic importance, such as MY. The ideal would be to increase FE and reduce CH₄ while maintaining appropriate levels of production.

2.5 CHALLENGES OF INCORPORATING METHANE EMISSIONS INTO BREEDING SCHEMES

Exploiting animal variation in methane production via animal breeding is an additional mitigation method that can be used, as it is economical, cumulative, and permanent (Hayes et al. 2013). Various studies have already been conducted or are under way for the implementation of genetic selection for reduced methane emissions in dairy cattle, but still they suffer from a small number of animals phenotyped for methane emissions to make a significant contribution to national breeding scheme (Chagunda et al. 2009; Garnsworthy et al. 2012b; Lassen and Lovendahl,

2016). A large phenotyped reference population is needed to achieve accuracy levels of estimated breeding values (**EBVs**) for a successful breeding program for reduced methane emissions.

In addition to the requirement of establishing a large enough phenotyped population, there are challenges due to the various phenotypic measurement techniques used and the alternative ways to express the methane emission trait, which poses uncertainty on what would be the best trait for selection (Hagemann et al. 2011). Incorporating methane emissions into selection indices based on this uncertainty is challenging, as the full impact of selecting for this trait is unknown. Measuring a trait on several thousand animals requires the trait to be easily recorded in a cost-effective manner. Thus, it is imperative to continue developing new direct or indirect measurement techniques that would help enhance the capability for reducing emissions through genetic selection.

Lastly, direct selection for reduced CH₄ emissions poses a challenge due to the measurement techniques being complicated and expensive (Pryce et al. 2014). This challenge may be overcome by using an indirect selection through other traits that would increase FE. Egger-Danner et al. (2012) suggested that lowering RFI reduces methane emissions per day, paving the way for future research to be conducted to determine the relationship between methane emissions and definitions of FE traits. For these investigations to be possible, as mentioned earlier, a large enough reference population would need to be established. However, the number of phenotyped animals required for such a reference population to obtain high accuracy EBVs for methane emissions almost certainly exceeds the available resources. An international effort is necessary to overcome these constraints and bring together data on ruminant production, feed intake, and methane emissions. Therefore, to be able to move forward with a genetic evaluation and ranking of animals for methane emission collaboration is needed in terms of sharing ideas, experiences,

protocols, and phenotypes, and coming to a consensus regarding what phenotype to collect and implement in selection indices.

2.6 STANDARDIZATION OF NATIONAL METHANE EMISSION DATA SETS

Despite genomic selection simplifying the evaluation requirements for a particular phenotype, the number of cows recorded for methane emission within each country may still be too small to achieve an accurate national genomic estimated breeding value (Pszczola et al. 2012). International research initiatives are currently underway to implement feed efficiency and methane emissions in national breeding programs. A collaborative initiative's goal to deliver a multi-country database for FE and methane emission brought together Australia, Canada, Denmark, Switzerland, United Kingdom, and the United States. The project entitled Efficient Genome Dairy Project (**EDGP**) made possible the data exchange between partners, allowing for an increase in reference population size, improving genomic evaluations for these two novel traits. In addition, other European countries are also interested in developing EBVs for these efficiency related traits (ICAR and GenTORE). Such efforts have shown the value of integrating data to improve reference populations and enhance the accuracy of prediction of numerous traits in cattle.

Brito et al. (2018) carried out a meta-analysis of methane emission traits published in recent years. They concluded that methane emission traits are under moderate genetic control in cattle and sheep. It appears that direct measures of methane intensity and residual methane traits have consistent estimates of heritability across studies. However, the contrary was found across studies for methane production, methane yield, and indirect methane emissions based on predictive equations, which all exhibited significant heterogeneity across studies. This could have practical

implications when determining what trait definition to use for selection purposes, particularly when involving sharing of databases.

In addition to the challenge of combining phenotypes from different countries using different trait definitions, there is also the genotype-by-environment interaction to consider, which is defined as animals and their offspring's different performances in different environments than those where they were raised or selected (Falconer and Mackay, 1996). A solution could be using a multi-trait model as it can accommodate traits evaluated as separate traits in different environments (Calus and Veerkamp, 2011; de Haas et al. 2012), thereby addressing both the genotype-by-environment interaction and discrepancies in trait definitions appropriately. However, a challenge when considering the multi-trait models is that it is more complex and prone to overfitting, which may result in a loss of prediction accuracy relative to single-trait prediction (Runcie and Cheng, 2019). Therefore, careful consideration must be applied when fitting the model when using this approach despite being beneficial when combining the different data sources.

2.7 FINAL REMARKS

While ruminants have advantages in using otherwise unsuitable fibrous feed for production, they contribute to GHG emissions mainly due to enteric methane production. Genetic selection is one of several strategies for mitigating methane emissions in dairy cattle while maintaining the selection goal of maximizing milk production. It is possible to select for methane efficient animals; however, implementing selection for reducing methane emissions from dairy cattle into national breeding programs will require consideration of the various trait definitions, correlations with production traits, and optimal breeding goals. The selection of individuals with

lower than average CH₄ emissions should not compromise the current efforts towards improving efficiency of feed utilization and production levels. Therefore, the next steps to evaluate whether methane emission can be implemented in future breeding goals is to investigate potential methane traits (e.g., methane production, methane yield, methane intensity) and their genetic parameters to decipher which methane phenotype to select for.

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2.9 FIGURES

- 1) $\text{Glucose} \rightarrow 2 \text{ pyruvate} + 4\text{H}$
(carbohydrate metabolism);
- 2) $\text{Pyruvate} + \text{H}_2\text{O} \rightarrow \text{acetate} + \text{CO}_2 + 2\text{H}$;
- 3) $\text{Pyruvate} + 4\text{H} \rightarrow \text{propionate} + \text{H}_2\text{O}$;
- 4) $2 \text{ acetate} + 4\text{H} \rightarrow \text{butyrate} + 2\text{H}_2\text{O}$.

Figure 2.1. Fermentation process in the reticulorumen and hindgut. Sugars are fermented to VFA through multi-step pathways producing reduced equivalents

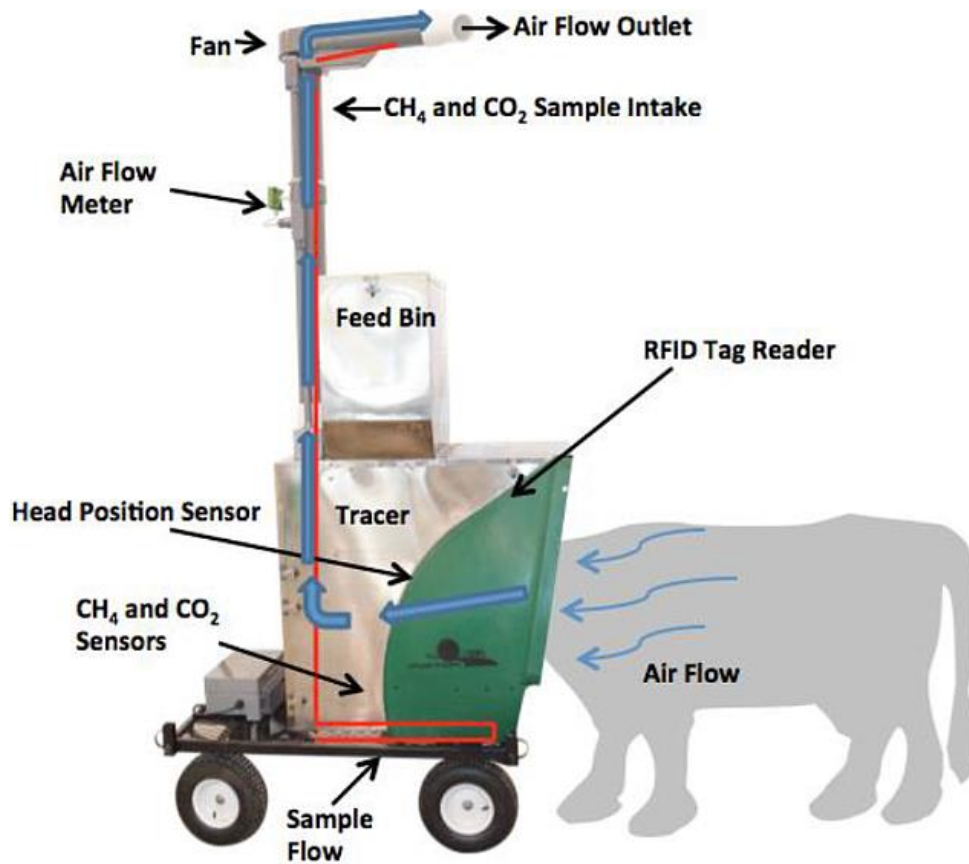


Figure 2.2. Layout of the GreenFeed (C-Lock Inc., Rapid City, SD) system used at University of Guelph (the flux method). RFID = radiofrequency identification (Hristov et al. 2015).

CHAPTER 3: ASSESSMENT OF METHANE EMISSION TRAITS IN CANADIAN HOLSTEIN COWS

3.1 ABSTRACT

Genetic selection is one of the best approaches to mitigating methane emissions from dairy cattle since methane emission related traits are heritable and genetic gains are persistent and cumulative over time. The objective of this study was to estimate heritability of alternate methane emissions phenotypes and the genetic and phenotypic correlations between them in Canadian Holstein cows. From 1,373 to 1,765 individual records were collected on 330 Holstein cows, depending on the trait, from two herds using GreenFeed machines. The average daily methane production was 463.5 grams per day with a standard deviation of 115.4. Three methane traits were analyzed in this study: the amount of daily methane produced (in g/d) measured over a span of 5 days (**MeP**), methane yield, defined as grams of methane per kg of dry matter intake (**MeY**), and methane intensity, defined as grams of methane per kg of milk per day (**MeI**). Genetic parameters were estimated using univariate and bivariate repeatability animal models, which included statistically significant fixed effects ($P < 0.05$) and random direct additive genetic, permanent environment, and residual effects. The fixed effects tested were contemporary group (CG; composed of herd, year of observation, and season of observation (2 classes), age of cow at calving (6 classes), and days in milk (3 classes)). The heritability estimates of 0.16 (± 0.10), 0.27 (± 0.12) and 0.21 (± 0.14) were obtained for MeP, MeY, and MeI, respectively. MeP and MeI were highly genetically correlated $r_g = 0.94$ (± 0.23) in this study, indicating that selection for daily methane production would also result in cows that would be efficient in methane emission per unit of

product. Moderate to high genetic correlations between the other pairs of methane traits were found indicating that selecting for improving one trait would also improve the others. The overall Spearman rank correlations between estimated breeding values for methane emission traits ranged from 0.88 to 0.90. However rank correlations for sires with phenotyped daughters ranged from 0.64 to 0.67, indicating substantial sire re-ranking and impact on selection decisions. Overall, the results of this research suggest that there is potential to mitigate methane emission in Canadian Holstein cattle through genetic selection and they provide the initial genetic parameter estimates needed for the genetic evaluation of methane emission traits in the Canadian Holstein population. The methane emission trait of choice will depend on how it will possibly be included in the genetic evaluation program.

Keywords: Holstein cattle, genetic parameters, methane, selection

3.2 INTRODUCTION

The global population is rapidly increasing, and environmentally sustainable food production is also becoming a growing topic of discussion. The agricultural industry is often targeted for its contribution to environmental footprint and, particularly, the environmental impact of livestock production has been criticized. Although dairy cattle represent only a fraction of the total green-house gas (**GHG**) emissions, increased awareness and consumer pressure to implement initiatives to improve efficiency and sustainability have pushed the Canadian dairy industry to investigate ways to mitigate its environmental footprint. In 2016, Canada joined 195 signatories in one of the global initiatives to lower GHG emissions, the Paris Climate Agreement. By signing this agreement, Canada has committed to decreasing emissions by 30% of 2005 levels by 2030 (National Inventory Report, 2019; Environment and Climate Change Canada, 2020). Although the inventory of livestock per sector has decreased since 1990, emissions within the sector have increased by 28% between 1990 and 2017 and Canada's agricultural emissions have only decreased 1% since peak levels in 2005 (National Inventory Report, 2020). In 2017, emissions of methane from livestock accounted for 3.9% of Canada's GHG emissions, a 13% increase since 1990 (National Inventory Report, 2019). Therefore, mitigating methane emissions from dairy cattle industry is of interest.

Several systems for measuring direct methane production have been available, such as the automated head-chamber system (e.g., GreenFeed machines), the sulfur hexafluoride (**SF₆**) tracer gas system, and respiration chambers. Studies compared all three systems concluding that the daily

methane production measured by Greenfeed machines was similar to the values obtained using SF₆ and respiration chambers (Hammond et al. 2016; Huhtanen et al. 2019). Research groups in Spain have also put considerable effort into comparing methods of recording methane emissions. Both non-invasive direct measures (e.g., non-dispersive infrared methane detector devices) and proxies of methane (e.g., rumination time) have been investigated and corresponding genetic parameters estimated (López-Paredes et al. 2020).

Countries have investigated methane emission traits to be implemented in their national breeding schemes. Denmark, Netherlands, Spain, and United Kingdom have estimated the genetic parameters of methane emission traits (de Haas et al. 2011; Lassen and Løvendahl 2016; Breider et al. 2019; López-Paredes et al. 2020). These studies have reported genetic variation in cattle methane emissions. Heritability estimates ranging from 0.12 to 0.45 depending on the methane trait analyzed and the stage of lactation (e.g., methane intensity, defined as g of methane emitted over a week per fat and protein corrected milk and early to mid or late lactation). In the United Kingdom, research has suggested that a reduction in milk volume (and increase in milk solids), body weight and feed intake, and improving fertility and longevity could reduce emissions by requiring fewer replacements, while maintaining production levels (Bell et al. 2015). Another approach suggested by Pryce et al. (2020) was the use of residual methane, which is defined as the difference between actual methane and predicted methane, which is obtained regressing on traits such as milk yield, body weight, and dry matter intake (**DMI**). In this case, the desired estimated breeding value (**EBV**) would be from animals that emit less methane than that expected for their milk production level, body weight, and DMI.

González-Recio et al. (2020) suggested that a decrease in methane emissions can result from selecting for milk production over time, due to the associated reduction in cow inventory. Manzanilla-Pech et al. (2020) proposed using a multiple-trait approach, which included body weight, energy corrected milk, and methane production, due to higher prediction accuracies when compared to single-trait models for methane production in Danish dairy cattle. Therefore, there are many proposed technologies and methods in the literature thus far for mitigating methane emissions.

The Canadian dairy industry had always aimed to improve production by using superior genetics, feed and management practices. However, today an average dairy cow produces more GHG, due to a 20% increase in per-animal emissions since 1990, despite the decline in the dairy cattle inventory (National Inventory Report, 2019). There are two selection indices used in Canada: Lifetime Performance Index (**LPI**) and Pro\$ index, and neither contain a sustainability or efficiency trait (Canadian Dairy Network, 2015). Therefore, there is an opportunity to further enhance Canadian dairy cattle's production performance by including a means for mitigating methane emissions into Canadian dairy breeding program. The objective of this study was to investigate potential methane emission traits (methane production, methane yield, methane intensity) with respect to their genetic parameters and ability to rank selection candidates to help choosing the best trait to be considered in the Canadian dairy industry breeding program.

3.3 MATERIALS AND METHODS

3.3.1 Data Collection

Data used in this study were collected through the Efficient Dairy Genome Project (<https://genomedairy.ualberta.ca>), a global initiative investigating the use of selective breeding and novel genomics technologies to improve feed efficiency and reduce methane emissions in dairy

cattle. Data from two Canadian research stations located in Alberta and Ontario were used in the analyses. The data collection in these two stations is described next.

3.3.1.1. Ontario Dairy Research Centre

The first herd comprising the Canadian data was the Ontario Dairy Research Centre (**ODRC**) of the University of Guelph in Elora. In general, animals were housed in a free stall barn where they were fed, milked, and managed as the same as the rest of the milking herd. At approximately 120 days in milk (**DIM**) - 150 DIM, cows were moved from the main barn to a separate tie-stall wing within the barn in their first lactation. Cows were moved in groups ranging from two to four animals to minimize stress and brought into the tie-stall area three days before testing to allow for acclimatization and to be trained to the GreenFeed machine (GreenFeed; C-Lock Inc., Rapid City, SD). During the week that animals were in the tie-stall area, animals were fed total mixed ration (**TMR**) *ad libitum* which was delivered daily at approximately 11:00 h. After the training period, methane emissions were measured four times daily over the following five days (Monday to Friday) at 08:00, 12:00, 16:00, and 20:00 h. Each measurement was approximately 10-12 minutes long, with a minimum of 10 minutes (approximately 5 min of automatic drops 5 min of manual) of data collection for a test to be complete. The GreenFeed machine was adjusted so that each cow could receive approximately 30 or more drops (23 s apart among each drop) of high fat pellets from the overhead hopper per visit. The GreenFeed machine identifies animals through the use of their unique radio frequency identification (**RFID**) tag and estimates daily methane production (**DMP**, g/day) by measuring gas concentrations and airflow. Each animal's visit is determined based on the infrared sensors, and all data is automatically recorded. Cows were milked twice daily in their stalls at 05:30 and 17:30 h.

3.3.1.2. Dairy Research and Technology Center

The second herd comprising the Canadian data was the Dairy Research and Technology Center (**DRTC**) of the University of Alberta. During the methane emission recording period, all cows ranging approximately 30 DIM – 250 DIM were housed individually in a ventilated tie-stall barn with free access to water, and fed TMR *ad libitum* which was delivered daily at approximately 08:00h. Cows had access to an exercise area (an open dry lot) for 3 hours every second day. Initially (2016-2019), methane emission measurements were collected in groups/batches (10-15 cows/batch) of mixed parity lactating cows however, in this study only those that were in their first lactation were considered. Each group was measured for 12 days (twice a day, 12 hours apart). The first-day measurement started at 01:00 h, and 13:00 h, then shifted every day by 1hr to cover the 24h cycle by 12 days of recording. However, to keep recording consistent between herds, the protocol was changed to measuring three times a day (08:00 h, 12:00 h, 16:00 h) as described for the ODRC herd. During the methane testing period, the cows' group was arranged in a row in individual tie-stalls and the GreenFeed machine was moved to the cows. Each measurement took approximately 10 minutes (approximately 5 min of automatic drops 5 min of manual) to get a measurement from a single cow. The GreenFeed machine was adjusted so that each cow could receive six drops (40 s apart among each drop) of barley grain from the overhead hopper per visit. To allow a measurement to be recorded from a specific visit, the cow visiting the GreenFeed system needed to keep her head in an appropriate head position for 3-5 minutes. Cows were milked twice per day at 03:00 h and 15:00 h.

3.3.2 Variation in methane testing

Potential differences in methane emission among recording times were tested using R software (R Core Team, 2009) via the VAR.TEST and T.TEST functions, where F-tests and a two-

sided Welch's t-test, which assumes unequal variances between the groups, were conducted to test the differences in variances and means between the different measurement time periods, respectively. This was done to evaluate whether there were peak times and more variation in methane outputs possibly due to diet or management practices. This analysis was only applied to the ODRC herd, as the DRTC herd had followed a different protocol for approximately two and a half years prior to establishing set time periods for testing. Differences were considered significant at 5% probability.

3.3.3 Data set and Methane Traits

A total of 2,469 methane emission records were collected on 422 Holstein cows from August 2016 and March 2020 from the two research herds, i.e., ODRC and DRTC, with ODRC consisting of 272 cows and DRTC with 58 cows. Records ranged from 30 to 247 DIM from cows who calved between 22 and 33 months old. Records below 127 g/d (Quartile 1-1.5*Interquartile Range) and values above 799 g/d (Quartile 3 +1.5*Interquartile Range) were considered as outliers and removed from the dataset. Subsequently, only two outliers were removed. Cows were required to have at least three measurements to be kept in the data, as both herds collected methane measurements more than once per cow to assess variability. The pedigree file used for all methane traits in the analysis included 1,434 animals, consisting of 892 dams and 253 sires.

There are various methane emission traits used in livestock species (Herd et al. 2013; Brito et al. 2018). This study focused on three main methane traits:

- i. Methane production (**MeP**): grams of methane produced per day;
- ii. Methane yield (**MeY**): grams of methane produced per kg of DMI;
- iii. Methane intensity (**MeI**): grams of methane produced per kg of milk per day.

3.3.4 *Statistical analysis and Connectedness*

The DESCRIBE.BY function of the R-package “psych” in R software (R Core Team, 2009) was used for the descriptive analysis (Table 1). A linear fixed model was used to define the significant fixed effects ($P < 0.05$) to be included in the genetic analysis. The final model for the methane traits included the following fixed effects:

1. Age of the cow at calving split into 6 classes, which were ≤ 23 , $24 \leq \text{age} < 25$, $25 \leq \text{age} < 27$, $27 \leq \text{age} < 29$, $29 \leq \text{age} < 31$, and > 31 months. In both herds age of the cows at calving ranged from 22 to 33 months.

2. Days in milk (DIM) split into 3 classes, which were ≤ 134 , ≥ 135 & ≤ 155 , and > 155 days. Days in milk at DRTC ranged from 30 to 247 DIM, while at ODRC it ranged from 118 to 196 DIM.

3. herd-year-season was included in the model for MeP and MeI and only herd-year for MeY. Herd-year was used as the contemporary group for the trait MeY to lower the standard error of the genetic parameter estimates. Season was split into two classes, which were March-August and September-February.

Classification of the levels of fixed effects was based on the distribution of the data and the limitations imposed by the sample size.

Connectedness between contemporary groups (herd-year-season or herd-year in this study) was verified using the AMC program (Roso and Schenkel, 2006), which takes into account the number of genetic links between contemporary groups (CGs). From one to two CGs were disconnected depending on the methane trait and were removed from the dataset.

3.3.5 Variance components

Variance components were estimated using the Average Information Restricted Maximum Likelihood (**AIREML**) methodology and a repeatability animal model for MeP, MeY, and MeI. All analyses were performed using the ASReml software (Gilmour et al. 2015). The univariate animal model and the variance-covariance structure used are described below:

$$y_{ijklmn} = \mu + ADC_i + CG_j + DIM_k + a_l + pe_m + e_{ijklmn} \quad [1]$$

$$V \begin{bmatrix} a \\ p \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I\sigma_{pe}^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

Where y_{ijklmn} is the phenotype for MeP, MeY, or MeI ; μ is the overall mean; ADC is the fixed effect i th age of dam at calving class (6 classes); CG is the fixed effect j th herd-year-season of recording (10 classes: MeI; 12 classes: MeP) or j th herd-year of recording (6 classes: MeY); DIM is the fixed effect k th days in milk class (3 classes); a_l is the random additive genetic effect of the l th animal, distributed as $N(0, A\sigma_{2a})$, in which A is the relationship matrix and σ_{2a} is the additive genetic variance, pe_m the random permanent environmental effect of the m th animal with record, distributed as $N(0, I\sigma_{2pe})$, in which I is an identity matrix and σ_{2pe} is the permanent environmental variance; and e_{ijklmn} is the random residual effect n th observation (y_{ijklmn}).

Bivariate models were used to estimate genetic, permanent environment, and phenotypic correlations using the same effects as in the univariate model. Phenotypic correlations were estimated as $r_p = \frac{\sigma_{pj,pk}}{\sqrt{\sigma_{pj}^2 \sigma_{pk}^2}}$ where $\sigma_{pj,pk}$ is the phenotypic covariance between trait j and trait k , and

σ_{2pj} and σ_{2pk} are the phenotypic variance estimates for trait j and trait k , respectively. Similarly, genetic correlations were estimated as, $r_g = \frac{\sigma_{aj,ak}}{\sqrt{\sigma_{aj}^2 \sigma_{ak}^2}}$ where $\sigma_{aj,ak}$ is the additive genetic

covariance between trait j and trait k, and $\sigma_{aj}^2\sigma_{ak}^2$ are the additive genetic variance estimates for trait j and trait k, respectively. Lastly, permanent correlations were estimated as $r_{pe} = \frac{\sigma_{pej,pek}}{\sqrt{\sigma_{pej}^2\sigma_{pek}^2}}$ where $\sigma_{pej,pek}$ is the permanent environment covariance between trait j and trait k, and $\sigma_{pej}^2\sigma_{pek}^2$ are the permanent variance estimates for trait j and trait k, respectively.

The heritability (h^2) and repeatability (r) estimates from the univariate models were obtained as:

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2}, r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_p^2}$$

where σ_a^2 is a additive genetic variance, σ_p^2 is a phenotypic variance, σ_{pe}^2 is the permanent environmental variance.

Heritability of the average of a different number of methane emission records (from 2 to 5) were obtained as follows:

$$h_n^2 = \frac{h^2 \times n}{1 + (n - 1) \times r}$$

where h^2 is the heritability estimated from model [1], n is the number of measurements from 2 to 5, and r is the repeatability estimated from model [1].

3.3.6 Rank correlations and accuracy of estimated breeding values

Estimated breeding value (EBV) rank correlations between methane traits were used to assess potential re-ranking, considering either all animals or only sires with daughters with records for the methane traits. The Spearman rank correlations were obtained using the COR.TEST function in R software (R Core Team, 2009). In addition, the average accuracy of sires with daughters with records for the respective traits were estimated as:

$$accuracy = \sqrt{\left(1 - \frac{S^2}{(1 + F) \sigma_a^2}\right)}$$

where, S is the standard error of the EBV, and σ_a^2 is the population additive genetic variance and F is the inbreeding coefficient of an animal.

3.4 RESULTS AND DISCUSSION

3.4.1 Descriptive Statistics

Descriptive statistics for all traits (MeP, MeY, and MeI) used in the analyses are presented in Table 1. Means of MeP, MeY, MeI were 463.54 g/d, 23.46 g/kg and 14.61 g/kg, respectively. Means of the methane traits from the ODRC herd were larger than those from the DRTC herd. This could be explained by higher average body weight (+ 18 kg) of ODRC cows compared to DRTC cows). The average daily estimated methane production in this study is marginally higher than some findings in the literature using other methodologies. For example, Grainger et al. (2007), who measured methane on a small sample of Australian Holstein cows kept under experimental conditions, indicated an average daily methane emission of 331 g when measured using the SF₆ tracer method and 322 g when using respiration chambers. However, Huhtanen et al. (2015) and Denninger et al. (2019), who also used GreenFeed machines in Holstein dairy cows, showed average emissions of 447g/d and 426g/d. Means for the traits MeY and MeI reported in this study were similar to results found in literature (Lassen and Lovendahl 2016; Niu et al. 2018; Huhtanen et al. 2019). Niu et al. (2018) reported an average methane yield of 21.6 g/kg and methane intensity of 13.5g/kg using an international dataset of various recording methods. Also, Huhtanen et al. (2019) found an average methane yield of 21.6 kg/d and Lassen and Lovendahl (2016) reported an average of 8.61g/L for estimated methane intensity using Fourier-transform infrared spectroscopy technique during milking in an automated milking system (AMS). Lastly, the coefficient of variation (CV) of all traits ranged between roughly 20 and 35% across herds.

Therefore, substantial individual variation exists for all three methane traits indicating potential for genetic selection.

3.4.2 Variation over Time

F-tests and Welch two sample t-tests were conducted to test the differences in variances and means between the different recording times (08:00 h and 12:00 h, 16:00 h, and 20:00 h) for the ODRC herd. It was observed that the variances between test time 12:00 h and 20:00 h, and test time 16:00 h and 20:00 h were significantly different ($P < 0.05$). In addition, the mean methane production of all test times were significantly different ($P < 0.05$) from each other, except between test times 12:00 h and 16:00h (Figure 1). When looking into this, it appears that the highest amount of methane is emitted after feeding, going from 480 grams/cow to 515 grams/cow from 08:00 h to 12:00 h, as feeding takes place approximately an hour before. These results were in agreement with the literature. Hristov et al. (2015) who also used GreenFeed to study methane emissions, investigated diurnal methane emissions from dairy cattle fed once daily and found large variability between two different time points and increased methane emitted after feeding. In addition, Pszczola et al. (2017) also observed this pattern of methane emission levels increasing after feeding events and dropping over the night period. It would be expected that, if DRTC's diurnal patterns were analyzed, a similar pattern would have been observed based off their management practices with a peak showing after their feeding time. In the future, adjusting the data for the time of day at which the measurements are recorded should be considered, especially depending on the type of measurement equipment used, such as an AMS (Pszczola et al. 2017). If measurements are taken at times of the day with a peak (or low) methane level, the results could be skewed if time of the measurement are not included in the model. The approach in this study was to use the average of the four measures to represent the daily methane emission of a cow.

3.4.3 Genetic parameter estimates

Estimated variance components, heritability (h^2), and repeatability (r) for the traits are shown in Table 2. The estimated h^2 and corresponding standard error (**SE**) for MeP, MeY and MeI were 0.16 (0.10), 0.27 (0.12) and 0.21 (0.14), respectively. Despite the large standard errors, the heritability estimated for MeP was similar to that previously reported by Breider et al. (2019) using random regression in a bivariate analysis with milk yield or body weight (0.15) and van Engelen et al. (2018) for predicted methane production based on milk mid-infrared spectra (0.17). However, other studies have also reported more variable estimates for methane production, such as López-Paredes et al. (2020) who reported a lower heritability of 0.12 using a nondispersive infrared methane detector to calculate methane production from methane concentrations using an equation derived from Chagunda et al. (2009). Higher methane production heritability estimates ranging from 0.23 to 0.35 using other methodologies, such as direct measurement using the SF₆ method and predicted measurements (Manzanilla-Pech et al. 2016; Lassen and Lovendahl 2016; de Haas et al. 2011). The heritability estimates in this study of the ratio methane traits, MeY and MeI, were lower than those reported by Manzanilla-Pech et al. (2016), i.e. 0.30 for MeY and 0.42 for MeI, using genomic information. However, the current estimate for MeI was the same as that reported by Lassen and Lovendahl (2016) (0.21) and for both MeY and MeI the estimates found in this study were similar to those reported by van Engelen et al. (2018) (0.21 and 0.18, respectively). In a meta-analysis, Brito et al. (2018) reported a range of average heritability estimates from 0.19 to 0.24 across all three methane traits in a total of 18 peer-reviewed papers, including estimates from both sheep and cattle. Therefore, the estimates obtained in this study are close or within range to what has been reported in ruminants and, thus, show that methane emission traits are moderately heritable and could respond well to genetic selection.

Genetic selection for a trait has the potential to make cumulative, lasting changes in the population. Both Gonzalez et al. (2020) and Özkan Gülzari et al. (2018) examined various scenarios for integrating enteric methane into the dairy cattle breeding objectives to determine the genetic and economic response for traits in the selection index. They reported that total greenhouse gas emissions may not decrease, but methane intensity per unit of product may be lowered while maintaining the genetic selection goal of enhancing production and profit per herd. Quinton et al. (2018) have showed that genetic improvement in efficiency traits can also cause a decrease in beef cattle GHG emissions. Koenen et al. (2013) concluded that animal breeding strategies thus far for improved production efficiency can indirectly reduce emissions due to smaller herd sizes with higher production. This suggests that there is an opportunity for reducing methane emissions whilst still being able to maintain and improve production traits in dairy cattle over time. Furthermore, although methane emission is under some genetic control, the surrounding environment is the strongest determining factor (Lassen and Difford 2020).

Permanent environmental variances were large for methane traits, leading to moderate repeatabilities (0.54 (0.03), 0.49 (0.03) and 0.74 (0.02) for MeP, MeY and MeI, respectively, Table 2) meaning that all traits would benefit by repeated records, especially MeY, which had the lowest repeatability. Repeatability is an indicator of the correlation of two phenotypic observations taken on the same animal (Nakagawa and Schielzeth, 2010). Therefore, it is the upper limit of the trait's heritability (Falconer and Mackay, 1996). The repeatability estimates found in this study are within the range of values previously reported in the literature for methane production (from 0.36 to 0.79) (Bell et al. 2014; Haque et al. 2015; Manafiazar et al. 2016; López-Paredes et al. 2020).

Using the GreenFeed system allows more animals to be recorded over extended periods of time compared to respiratory chambers (McLean and Tobin, 1987) or the SF₆ tracer method

(Johnson et al. 1994). However, the ideal number of days an animal should be measured to best capture its genetic merit is still unknown (Manafiazar et al. 2016). The expected heritability of the average of methane production traits over 2 to 5 days was calculated and are presented in Figure 2 along with the heritability of a single day. As expected, the results show that MeY benefits considerably more from an increased number of days, with heritability estimates going from 0.27 to 0.46, while MeI and MeP estimates went from 0.21 to 0.27 and from 0.16 to 0.25, respectively. In addition, both MeI and MeP would not benefit from additional measurements after 5 days, given that their response curve flattens after 3 measurement days. On the contrary, MeY would benefit from more measurement days, but its heritability with 5 measurement days is already quite high (0.46), perhaps not justifying additional recording. The higher response of MeY may be explained by the fact that MeY is a ratio trait that accounts for DMI. Dry matter intake is affected by a multitude of factors and is highly variable daily (Negussie et al. 2019).

Heritability estimates for DMI have been shown to vary throughout lactation (Koenen and Veerkamp, 1998; Berry et al. 2007; Buttchereit et al. 2011). This same pattern is observed with methane emissions, where heritability estimates fluctuate throughout lactation (Pszczola et al. 2017). In this study cows were measured for methane emission in mid lactation (120 to 150 DIM) for a period of 5 consecutive days, as part of a larger project that aims to increase feed efficiency after the pick of lactation, i.e. after 60 DIM.

Additionally, it is worth noting that despite the value added through repeated measures, the tests could be separated by 3 to 14 days. When short-term measurements are used, repeatability is lower, likely due to differences in the duration and volume of feed consumed prior to testing. Thus, to resolve this issue short-term measurements of methane emissions could include more measures (between 3 and 20) over an extended period of time (Pickering et al. 2015). This needs further

investigation, but it will be less practical and feasible when considering application in a breeding program.

3.4.4 Correlations among methane traits

Phenotypic and genetic correlations between all methane traits are shown in Table 3. Genetic correlations (SE) between MeP and ratio methane traits MeY and MeI were estimated to be 0.73 (0.26) and 0.94 (0.23) with permanent and residual correlations of 0.69 (0.12) and 0.64 (0.02) between MeP and MeY, and 0.45 (0.15) and 0.91 (0.005) between MeP and MeI, respectively. Estimated genetic correlation between the ratio methane traits MeY and MeI was 0.68 (0.23) with permanent and residual correlations of 0.71 (0.14) and 0.63 (0.02). Estimated genetic correlations had large standard errors due to the relatively small dataset used in this study.

Previous literature reporting correlations between different methane traits are limited in dairy cattle. However, Kandel et al. (2017) reported high genetic correlations between MeI and MeP predicted from mid-infrared milk spectra (0.71), which is similar to those obtained in this study. Donoghue et al. (2016) reported a lower genetic correlation of 0.50 between MeP and MeY in Angus beef cattle. Manzanilla-Pech et al. (2016) also investigated methane phenotypes in Angus beef cattle and found genetic correlations of 0.62 between MeP and MeY, 0.18 between MeP and MeI, and 0.86 between MeY and MeI. Similarly, Van Engelen et al. (2018) recently reported high genetic correlation (0.63) between MeP and MeY and low (0.20) between MeP and MeI with high standard errors (0.15-0.28) using milk mid-infrared predicted phenotypes. However, unlike the estimate of 0.73 in this study, van Engelen et al. (2018) reported a negative genetic correlation (-0.21) between MeY and MeI. Therefore, there is a large variability in the estimated genetic correlation among methane traits, which stress the importance of estimating these parameters in own populations and using larger datasets.

3.4.5 Accuracy of EBVs and EBV Rank Correlations

Regardless of which methane trait is to be included in the selection program, the accuracy of selecting for top breeding candidates is important. Average accuracies were calculated for the sires with phenotyped daughters for each methane trait (Table 2). When comparing the average accuracies estimated per methane traits differences were small and average accuracies were low for all traits (from 0.35 to 0.41), being the highest for MeY (0.41) closely followed by MeP (0.39). These low accuracies are due to the small dataset analyzed. Accuracies will increase as the dataset enlarges by including data from international partners and the recording of methane emissions continues in Canada. Incorporation of genomic information into the genetic evaluation should also increase accuracy EBV for the methane traits. Therefore, sharing genotypes and phenotypes with other countries to increase the size of the reference population seems to be a useful alternative.

Spearman rank-order correlation coefficients were estimated to determine the degree of re-ranking that may occur due to the use of ratio traits MeY and MeI, accounting for DMI or MY, respectively, or using MeP (Table 4). Considering all animals, ranking correlation ranged from 0.88 to 0.90. However, rank correlations between the EBV sires ($n= 103$) with phenotyped daughters were much lower, being the highest between MeP and MeI at 0.67. The rank correlations between MeP and MeY and MeY and MeI were 0.65 and 0.64 respectively. Therefore, a large re-ranking of sires was observed. This indicated that sires, which are the best for reducing a methane trait of the daughters, may not necessarily be the best for the other methane traits, however a moderate to high genetic correlation between the methane traits exist, allowing for substantial indirect response to selection.

Therefore, putting all together, it seems that the methane emission trait of choice will depend on how it will possibly be included in the genetic evaluation program. For example,

methane production might be the selected trait, if the methane emission evaluation would be integrated into a selection index that would also include production and feed intake traits. Manzanilla-Pech et al. (2020) reported higher accuracy for genomic breeding values using multi-trait analysis, including energy corrected milk and body weight, indicating that a selection index using multiple traits would be the best approach.

However, breeding to reduce methane emissions in dairy cattle using direct individual measurements in a selection index is still a challenge due to the high cost of recording methane in a large number of animals. Predicting individual methane production using statistical methods based on milk mid-infrared spectra (Denninger et al. 2020) can be an alternative to increase the number of animals with records. Negussie et al. (2017) suggested a combination of indicators (milk MIR) or indirect traits (e.g., body weight) highly correlated with methane production into an indirect index, in order to allow increasing available information, statistical power, and accuracy of prediction of the EBVs.

Methane production might be the trait to include in the selection index, including other traits such as dry matter intake, body weight, and milk in order to mitigate methane emissions in Canadian dairy cattle. In the future, it would be necessary to investigate the correlations between methane production and other traits in the Canadian breeding program to avoid unfavorable correlated responses.

3.5 CONCLUSIONS

Methane emission is a moderately heritable phenotype, regardless of the expression of trait considered, i.e., methane production, yield or intensity. This shows potential of methane emission traits to be implemented into future selection breeding schemes. This study also showed that

genetically selecting for reduced methane production will also indirectly reduce methane yield and intensity due to its moderate to high genetic correlations with those traits, but this conclusion should be taken with caution due to high standard errors. The overall rank correlations between estimated breeding values for methane emission traits were relatively high (>0.88). However, rank correlations for sires with phenotyped daughters were only moderate (~ 0.65), indicating substantial sire re-ranking and impact on selection decisions. The methane emission trait of choice will depend on how it will possibly be included in the genetic evaluation program. In order to increase the accuracy of estimated breeding values for methane emission traits, a much larger dataset will be necessary.

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3.7 TABLES

Table 3.1. Number of Holstein cows and records, mean, standard deviation (SD), minimum, maximum, and coefficient of variation (CV) of methane production (MeP), methane yield (MeY), and methane intensity (MeI).

Trait	Herd	# Cows	# Records	Mean	SD	Minimum	Maximum	CV (%)
MeP (g/day)	DRTC	58	433	355.6	92.2	138	674	26.0
	ELORA	272	1,332	498.6	99.4	224	799	20.0
	Total	330	1,765	463.5	115.4	138	799	25.0
MeY (g/kg)	DRTC	54	342	17.2	4.5	5.11	34.4	26.0
	ELORA	233	1,126	25.4	5.7	11.67	75.68	22.0
	Total	287	1,468	23.5	6.4	5.11	75.68	27.0
MeI (g/kg)	DRTC	21	176	10.6	3.7	4.08	26.21	35.0
	ELORA	244	1,197	15.2	3.61	6.14	29.65	24.0
	Total	265	1,373	14.6	3.93	4.08	29.65	27.0

Table 3.2. Estimated additive genetic (σ_{2a}) permanent environmental (σ_{2pe}) residual (σ_{2e}) variances, heritabilities (h^2), repeatability (r), and average accuracy of EBVs for methane production (MeP), methane yield (MeY), and methane intensity (MeI).

Trait	σ_{2a}	σ_{2pe}	σ_{2e}	$h^2 \pm SE$	$r \pm SE$	Average accuracy
MeP	1147.3	2735.58	3279.59	0.16 (0.10)	0.54 (0.03)	0.36
MeY	6.51	5.15	12.34	0.27 (0.12)	0.49 (0.03)	0.44
MeI	2.53	6.24	3.15	0.21 (0.14)	0.74 (0.02)	0.32

Table 3.3. Estimated additive genetic (above) and phenotypic (below) correlations with their corresponding (SE) between pairs of methane traits, i.e. methane production (MeP), methane yield (MeY), and methane intensity (MeI).

Trait	MeP	MeY	MeI
MeP	-	0.73 (0.26)	0.94 (0.23)
MeY	0.67 (0.02)	-	0.68 (0.23)
MeI	0.70 (0.03)	0.63 (0.03)	-

Table 3.4. Estimated EBV rank correlation for sires with daughters (above diagonal) and for all animals (below diagonal) between pair of methane traits, i.e. methane production (MeP), methane yield (MeY), and methane intensity (MeI).

Trait	MeP	MeY	MeI
MeP	-	0.65	0.67
MeY	0.90	-	0.64
MeI	0.88	0.88	-

3.8 FIGURES

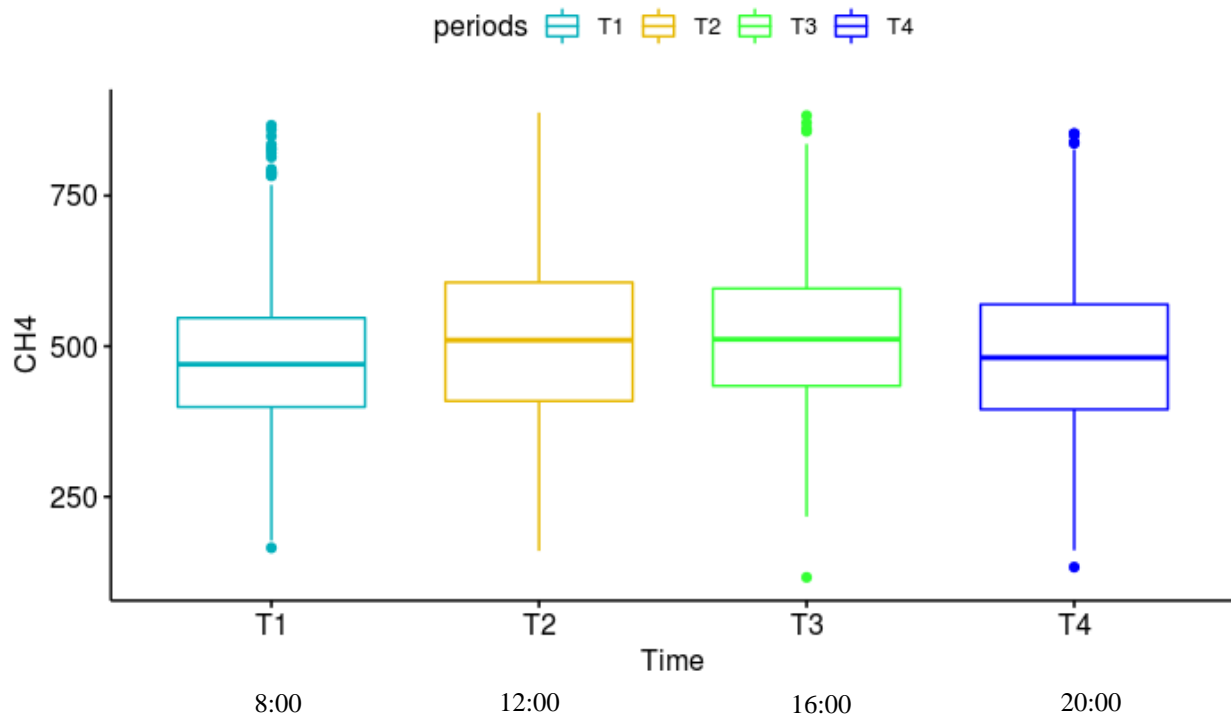


Figure 3.1. Daily methane production from dairy cows fed once daily, measured using GreenFeed. Means between T3 and T2 were not significantly different and variances between T1 and T3, as well as T3 and T4, were not significantly different ($P > 0.05$).

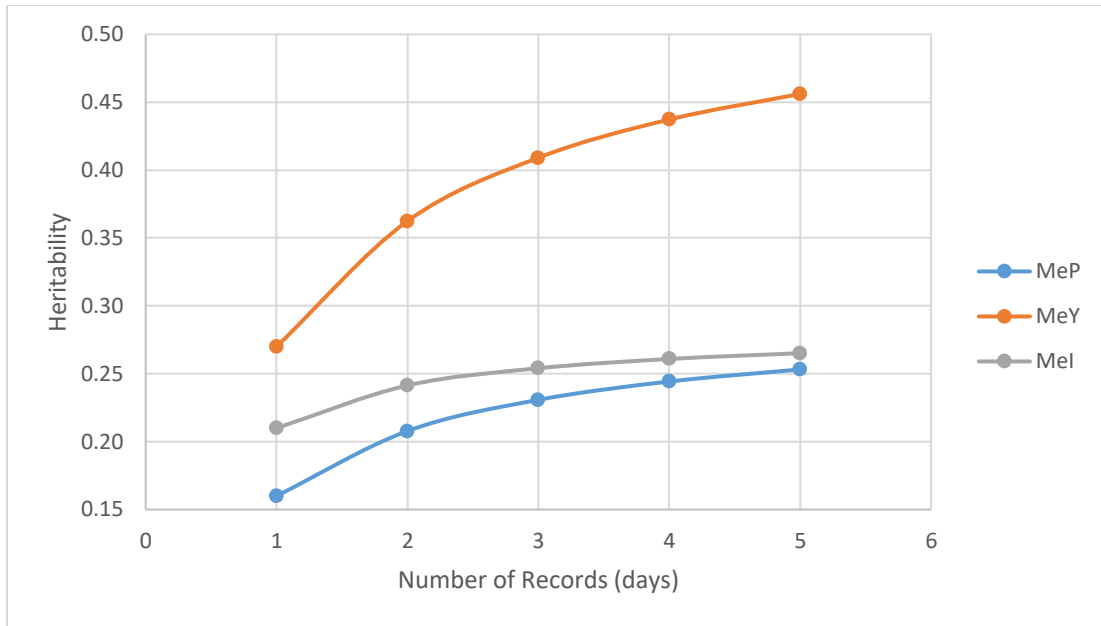


Figure 3.2. Heritability estimates for a single record and for the average of different numbers of methane emission records (n= from 2 to 5) for methane production (MeP), methane yield (MeY), and methane intensity (MeI).

CHAPTER 4: GENERAL DISCUSSION

4.1 FINAL REMARKS

Considering that consumers worldwide are increasingly demanding details on the carbon footprint of livestock production, such as dairy, reducing methane emissions is an important goal. Genetic improvement may play a key role in reducing ruminant contribution to greenhouse gas (GHG) emissions and will help to mitigate the negative effects of enteric methane emissions.

Despite increases in milk production through the management and genetic selection and decreased costs per unit of product over recent decades, dairy systems are still facing the challenge associated with environmental costs (Baskaren et al. 2009). For dairy cattle, up to 12% of the feed's gross energy is lost as methane production, an avoidable loss of energy that could otherwise be directed into more productive purposes (Wanapat et al. 2015; Negussie et al. 2017). Thus, finding ways to solve both losses of energy and methane emissions' environmental impact is important to the dairy industry's sustainability in the future. The combination of reducing methane emissions by genetic selection and improved management (e.g., feed supplementation, decrease in herd size) will play a vital role in addressing the dairy industry's methane emission footprint. Therefore, this thesis had the overall objective of investigating potential methane emission traits to be included in future breeding goals of the Canadian dairy industry.

The first and second chapters provided context and justification for the genetic improvement of methane emission traits, as well as a review of the different methodologies used to record methane emissions and previous literature reports on genetic parameter estimates of methane

emission traits. The review also highlighted the limitations of implementing genetic selection for reduced methane emissions in dairy cattle, which was also experienced in our study. Little had been reported about the genetics of methane traits in the Canadian dairy industry before completing this study. The findings of this research provide some of the framework information necessary for the genetic improvement of methane emission traits in the Canadian dairy industry.

In Chapter 3, genetic parameter estimates for methane production (**MeP**) and two methane ratio traits, i.e. methane Yield (**MeY**), and methane intensity (**MeI**), and their genetic, phenotypic, permanent environment, and residual correlations were investigated. Methane traits were found to be moderately heritable, with heritability estimates ranging from 0.16 ± 0.10 for MeP to 0.27 ± 0.14 for methane ratio traits. Moderate to high genetic correlations between all pairs of methane traits were found, indicating that selecting for improving one trait would also improve the others. Furthermore, differences in average accuracies estimated per methane trait were small and, in all cases, accuracies were low varying from 0.32 to 0.44 due to the limited dataset available for the analyses. Rank correlations between estimated breeding values (**EBVs**) for methane emission traits for sires with phenotyped daughters were moderate, indicating substantial re-ranking of sires and impact on selection decisions. Aggregating this information, it seems that the methane emission trait of choice will depend on how it will possibly be included in the genetic evaluation program. For example, methane production might be the chosen trait, if the methane emission evaluation would be integrated in a selection index that would also include production and feed intake traits.

The results of this study should be taken with caution due to the large standard errors of the estimates. However, the results presented here offer the first set of parameters needed to determine

how best to implement methane emission genetic evaluation in the Canadian dairy breeding program.

4.2 LIMITATIONS AND FUTURE RESEARCH STEPS

The methane data utilized in this research was collected from two research facilities in Ontario and Alberta and was used to make inferences about prospects for national genetic improvement of methane emissions in Canadian Holstein cattle. Therefore, it is assumed that parameters estimated for these research herds hold for commercial herds. As there is not a commercial herd recording methane emission in Canada and among the international partners, this assumption is needed, but should be tested in the future if commercial data becomes available.

Furthermore, the major limitation of this study was the limited number of animals with methane emission records available, which decreased the accuracy of the genetic parameter estimates and EBVs. One alternative proposed to increase sample size is to predict cow methane emissions with relatively high accuracy using milk mid-infrared (MIR) spectroscopy (Deharang et al. 2012; Vanlierde et al. 2015, 2016, 2018; Shetty et al. 2017). Similar to de novo synthesis of milk fatty acids, the production of methane is dependent on ruminal fermentation, thus several recent studies have investigated the prediction of individual methane emissions of dairy cows based on milk MIR spectra (Vanlierde et al. 2015, 2016, 2018). These studies reported cross-validation coefficients of determination ranging from 0.65 to 0.77 for MIR predicted methane emissions, using as gold standard values methane emissions measured using either respiration chambers or sulfur hexafluoride gas technique, suggesting that MIR predicted methane emissions could be used as proxies of the actual methane emissions in genetic evaluations (Vanlierde et al. 2015, 2016, 2018). However, for accurate prediction of cow methane emissions using milk MIR

spectra, a large reference population of cows with actual methane emission measurements is still needed, so collaboration for increasing the size of the reference population of cows phenotyped for methane emission is crucial yet.

As the number of data records collected in Canada increases and it is combined with the EDGP database data records from international partners, the model built in this research could be used to re-estimate parameters to improve accuracy and representativeness of estimates. Additionally, it will be useful to estimate genetic correlations between methane phenotypes and economically important traits for the Canadian dairy population in the future.

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