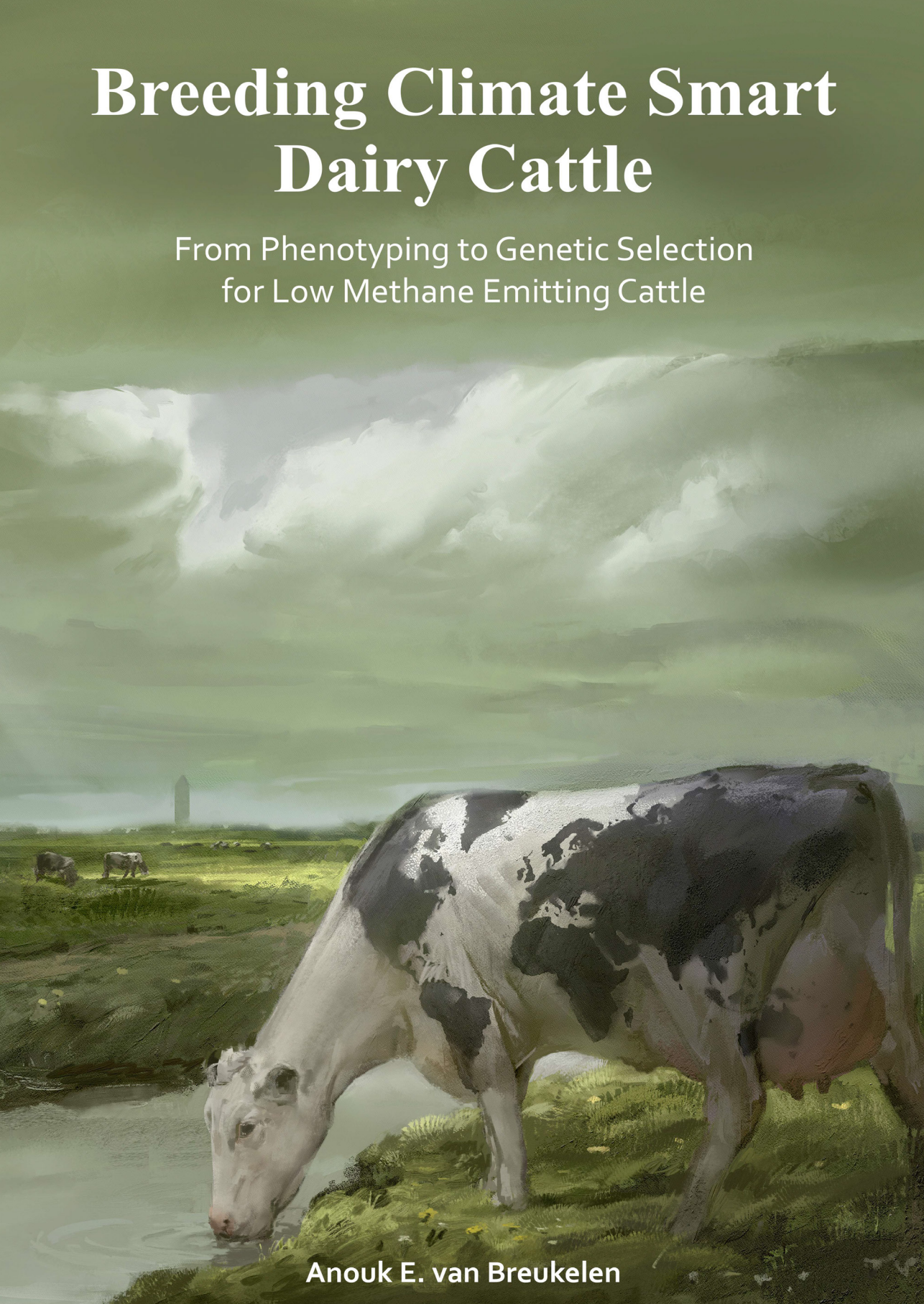
Breeding Climate Smart Dairy Cattle

From Phenotyping to Genetic Selection for Low Methane Emitting Cattle



Anouk E. van Breukelen

Propositions

1. Breath sensors placed in the feed bin of milking robots (i.e. sniffers) provide an inexpensive indicator for total methane production of individual cows.
   (this thesis)

2. The greatest impact in reducing methane emissions through animal breeding will be achieved by sharing data between institutes and countries.
   (this thesis)

3. It is a pitfall to assume that automated phenotyping in innovative PhD projects is automatic.

4. Informed decision-making in society is impossible without public understanding of science.

5. Repairability should be a key design criterion for technological devices.

6. Self-reflection is the key to tolerance.

7. AI's growth mirrors our own: rapid, unpredictable, and impactful.

Propositions belonging to the thesis entitled

Breeding climate smart dairy cattle: from phenotyping to genetic selection for low methane emitting cattle

Anouk E. van Breukelen

Wageningen, 15 October 2024

Breeding climate smart dairy cattle

From Phenotyping to Genetic Selection for Low Methane Emitting Cattle

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This research was conducted under the auspices of the Graduate School of Wageningen Institute of Animal Sciences (WIAS), the Netherlands

Breeding climate smart dairy cattle

From Phenotyping to Genetic Selection for Low Methane Emitting Cattle

Anouk E. van Breukelen

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr C. Kroeze,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 15 October 2024
at 3.30 p.m. in the Omnia Auditorium.

Anouk E. van Breukelen
Breeding climate smart dairy cattle: from phenotyping to genetic selection for low methane emitting cattle,
185 pages

PhD thesis, Wageningen University, Wageningen, the Netherlands (2024)
With references, with summary in English

DOI: https://doi.org/10.18174/671955

Abstract

van Breukelen, A.E. (2024). Breeding climate smart dairy cattle: from phenotyping to genetic selection for low methane emitting cattle. PhD thesis, Wageningen University, the Netherlands

Various strategies have been proposed to reduce enteric methane (CH_4) emissions from dairy cattle, targeting management, feeding strategies, feed additives, vaccination, and animal breeding. Among these, animal breeding currently shows the largest long-term potential, due to its low implementation costs, and the permanent and cumulative effects. Nonetheless, implementing CH_4 mitigation in breeding programmes is still in its infancy, limited by the lack of large-scale phenotyping of CH_4 emissions of individual cows to estimate sufficiently reliable genetic parameters and breeding values required for informed breeding decisions. However, recent innovations have accelerated the collection of CH_4 phenotypes. In this thesis, a novel dataset was collected measuring enteric CH_4 emissions of individual cows using 'sniffers' in the feed bin of milking robots. Finally, the dataset included 74,569 weekly mean CH_4 concentration (ppm) records on 7,139 cows from 68 commercial dairy farms. The research objectives in this thesis were to: 1) define a CH_4 trait from the raw concentration measurements, and estimate heritabilities and repeatabilities for these traits, 2) investigate the genetic relationship between two CH_4 recording methods (sniffers and GreenFeed units), 3) investigate the accuracy of different recording schemes and estimating the genetic correlations between lactation periods, and 4) investigate the relationships between CH_4 and other breeding goal traits. The defined phenotype for weekly mean CH_4 concentration measured by sniffers had a moderate heritability of 0.17 ± 0.04 and a repeatability of 0.56 ± 0.03. As the sniffers only measure concentrations, and not the total grams of CH_4 emitted by breath, genetic correlations were estimated between the weekly mean CH_4 concentration (ppm) phenotype and a weekly mean CH_4 production (g/day) phenotype from GreenFeed units. The genetic correlation was 0.76 ± 0.15, indicating that selection for lower CH_4 concentrations will result in a reduction of total CH_4 production output in g/day. Furthermore, the results confirmed that the genetic variance changed over a lactation and showed that a short CH_4 recording period during the first or last weeks of the lactation can result in lower genetic gains than predicted when assuming unity genetic correlations during lactation in a repeatability model. The genetic relationships among CH_4 concentration, DMI, body weight, and milk yield traits were weak: 0.06 ± 0.10 with dry matter intake, -0.04 ± 0.10 with body weight, and -0.04 ± 0.08 with milk yield for first parity cows. Overall, the results of this thesis are essential for the application of CH_4 emission recorded with sniffers for breeding programmes and will be used to develop breeding values estimation procedures and selection strategies needed to construct practical breeding strategies for more environmentally sustainable dairy farming in the Netherlands.

Contents

1

General introduction

To mitigate global warming, governments, research institutes, and industry worldwide, aim to reduce the emissions of methane (CH_4) which is a potent greenhouse gas. Ruminants are an important emitter of CH_4, which is mostly produced during enteric fermentation and emitted into the air by breath and belching (Herrero et al., 2016). Enteric fermentation occurs in the digestive system of ruminants, where organic material from feed is broken down by microorganisms that produce CH_4 in the process.

Dairy cattle are a key group of ruminants where CH_4 emissions should be mitigated. Various strategies have been put forward to reduce CH_4 emissions of dairy cows, which target management, feeding strategies, feed additives, vaccination, and animal breeding (Knapp et al., 2014). Of these, animal breeding shows large potential as implementation costs are low, the results are cumulative, and the effect is permanent. Nonetheless, implementing CH_4 mitigation in breeding programmes is still in its infancy. A limitation to practical application has been the lack of information on CH_4 emissions of individual cows. Information on many individual cows is needed to estimate sufficiently reliable genetic parameters which are required for informed breeding decisions (de Haas et al., 2017). However, recent innovations in methods to record enteric CH_4 emissions have accelerated the collection of CH_4 phenotypes.

This thesis describes a large-scale phenotyping project, where CH_4 concentrations from the breath of dairy cows were measured during milking in automated milking systems (AMS). From this novel dataset, genetic parameters have been estimated which are essential to form a basis for innovations in breeding for lower enteric CH_4 emissions. In this introduction, the necessity and urgency of innovations in reducing CH_4 emissions is described. It then provides insights into the methods for measuring enteric CH_4 emissions of individual dairy cows. Thereafter, the potential of animal breeding to reduce CH_4 emissions is highlighted, along with a description of innovations and methods that are needed to apply breeding strategies. The introduction concludes with the objectives and structure of the further chapters of this thesis.

1.1 Methane

1.1.1 The impact on climate change

Climate change, induced by the emission of greenhouse gases, is an urgent environmental issue (IPCC, 2013). Without greenhouse gases, all thermal radiation would be reflected, and the earth would become too cold to sustain life. However, an overabundance of greenhouse gases is causing the earth to warm (Figure 1.1), which in turn can cause a variety of other risks for human livelihood, such as occurrences of drought and other natural disasters (Rising et al., 2022). In the Paris Agreement, an international pledge has been made to keep global temperatures this century well below 2 degrees Celsius above pre-industrial levels, and to

pursue efforts to limit the temperature increase to no more than 1.5 degrees Celsius (UNFCCC, 2015). In response, many countries have set emissions reduction targets to adhere to limiting the global temperature rise to no more than 1.5 degrees Celsius. For the Netherlands, this means reducing emissions by 55% by 2030 compared to 1990 levels and achieving net zero emissions by 2050 (EZK, 2022).

One potent greenhouse gas targeted for mitigating the effects of global warming is CH_4 (UNEP and CCAC, 2022). The global warming effect of CH_4 is 27.2 times stronger than that of carbon dioxide (CO_2) over a 100-year period, as reported in the IPCC AR6 report (IPCC, 2021). On the other hand, CH_4 has a half-life of just 8.6 years, meaning that in 8.6 years 50% of CH_4 is converted into CO_2 and water vapour, whereas the majority of CO_2 remains in the atmosphere for several centuries. Consequently, by reducing CH_4 levels in the atmosphere, global warming effects can be quickly mitigated or even partly reversed when emissions of other greenhouse gases are simultaneously halted (Mitloehner et al., 2020).

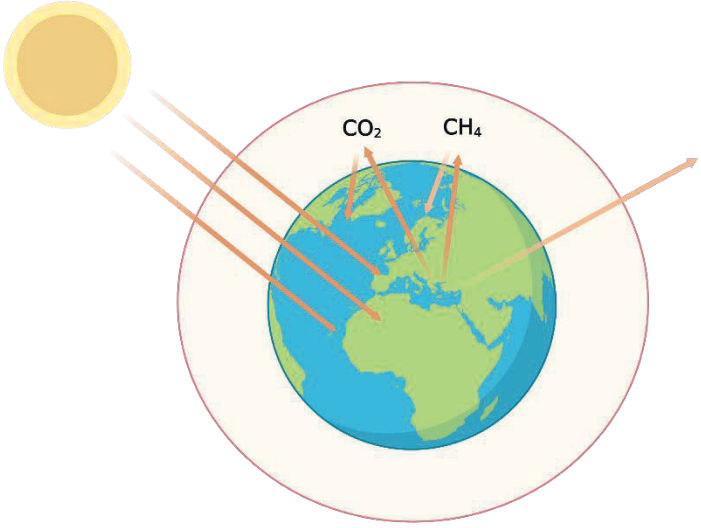

Figure 1.1. Reflection of thermal radiation by greenhouse gases, such as carbon dioxide (CO_2) and methane (CH_4), in the earth's atmosphere

Another reason why reducing CH_4 emissions is highly urgent is that during the conversion of CH_4 in the atmosphere the CH_4 molecules react with OH-radicals by which ozone (O_3) is produced (Tie et al., 1992). High concentrations of O_3 are detrimental to all forms of life, and the concentration of O_3 has doubled over the past century, due to the breakdown of CH_4. It is

estimated that worldwide, between 0.3 and 1.2 million people per year die as an effect of high O_3 concentrations, which affect the lungs and mucous membranes (van Dingenen et al., 2018). In addition, a decline of 4 to 12% of crop yield for the most important crops in the world has been estimated to be a result of increased O_3 concentrations (Mills et al., 2018). Thereby, O_3 forms a major risk for the world's food supply.

1.1.2 Methane emissions from livestock

Methane gas is produced during the decomposition of organic materials in environments with little or no oxygen. About half of all CH_4 emissions come from natural sources, such as: lakes, termites, marshes, wetlands, and the permafrost (Jackson et al., 2020). The other half comes from anthropomorphic sources, with the largest contribution coming from fossil fuels (± 18%) and livestock (± 17%), and smaller contributions coming from waste/landfills, wet rice cultivation, and biomass burning. As a result of human activities, global emissions of CH_4 and CO_2 have increased steeply since the commence of the industrial revolution and have increased from about 250 CH_4 molecules per billion parts of air to the current level of about 1,900 parts per billion (Figure 1.2).

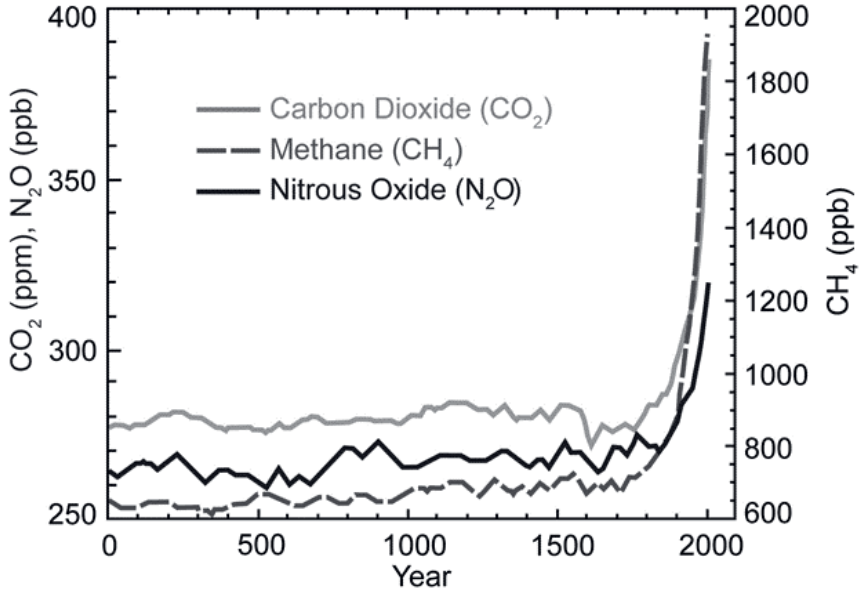

Figure 1.2. Atmospheric concentrations of greenhouse gases (carbon dioxide, methane, and nitrous oxide), from the year 0 to 2005. Increases since around the year 1750, can be attributed to anthropomorphic emissions. Source: https://www.ipcc.ch/site/assets/uploads/2018/02/ar4-wg1-chapter2-1.pdf

With a contribution of 17%, our livestock form a major component of global CH_4 emissions. In livestock, the majority of CH_4 emissions are produced by ruminants such as cattle, during

anaerobic fermentation by microbiota in the rumen (Herrero et al., 2016). Of the enteric emissions, around 97% is emitted through breath and belching (Munoz et al., 2012) and enteric CH_4 emissions have increased globally with 54.3% over the past 60 years (Patra, 2014). Furthermore, under current global trends, CH_4 emission from ruminants are expected to continue increasing, largely because the number of cattle and other ruminants in the world is also projected to increase (Robinson et al., 2011).

Enteric emissions are biogenic and part of short carbon cycles, where CO_2 produced is absorbed by plants, consumed by animals, and then re-fermented into CH_4 and converted back into CO_2 (Figure 1.3). However, relying on grassland carbon to offset ruminant emissions is not feasible, highlighting the urgency of reducing livestock emissions (Wang et al., 2023). Consequently, livestock emissions are considered anthropogenic, resulting from human activity rather than solely natural processes (IPCC, 2021). Additionally, by reducing the atmospheric pressure from livestock CH_4 being converted into CO_2, plants can be optimally used as a carbon sink for general anthropogenic emissions.

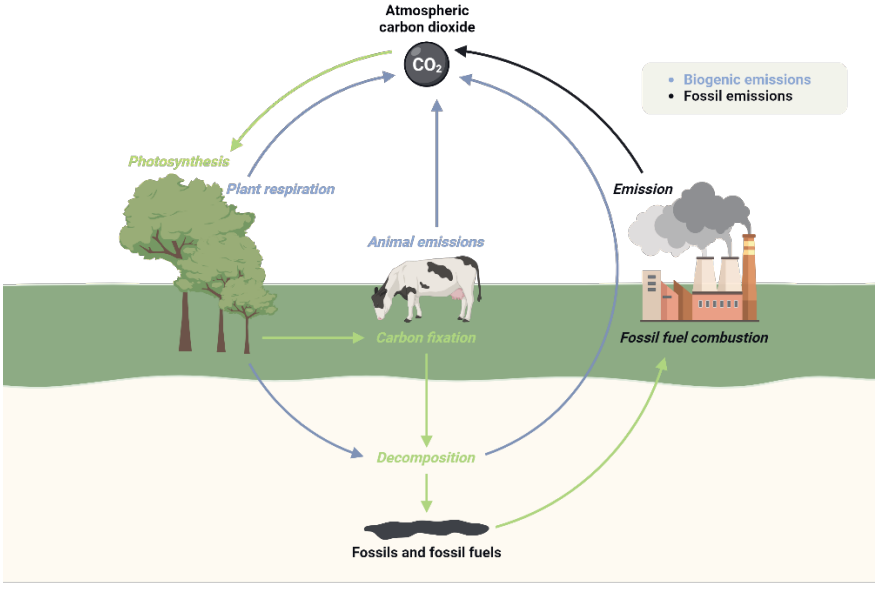

Figure 1.3. The short carbon cycle of biogenic carbon emissions and the long carbon cycle of fossil carbon emissions. When methane is converted in the atmosphere, carbon dioxide (CO_2) is formed which adds to the carbon cycles

Previous research has shown that individual dairy cows differ in the amount of enteric CH_4 emissions they emit, and that part of this variation between animals is heritable (Lassen and

Difford, 2020). With the initial estimated genetic parameters, a simulation study by de Haas et al. (2021) estimated the potential of using animal breeding to mitigate CH_4 emissions of dairy cows. The authors estimated that with the current breeding goal, individual animal CH_4 emissions would increase by 13% up to the year 2050. However, because in the current breeding goal milk production is projected to increase, CH_4 emissions per kg of milk produced (i.e., methane intensity) are expected to decrease by 13%. But with additional genetic selection on CH_4 emissions, the CH_4 emissions per kg of milk could reduce by up to 24% by 2050.

Application of CH_4 mitigation by animal breeding outside of research and in industrial breeding programmes is still in its infancy. Over the past years, various countries, including the Netherlands, have focussed on collecting enteric CH_4 measurements on hundreds to thousands of individual cows to be able make informed breeding decisions in the future (Manzanilla-Pech et al., 2021). However, the new phenotypes are still to be implemented in practical breeding strategies in the Netherlands. To realise practical implementation, statistical models must be developed that estimate genetic parameters, and later the breeding values for CH_4 for all commercial dairy bulls. Thereafter, selection indices can be developed that encourage CH_4 mitigation while maintaining production, and without negatively impacting reproduction, health and welfare, conformation, and feed intake.

1.2 Recording Methane Emissions of individual cows

1.2.1 Methods of recording

To make informed breeding decisions, phenotypes for CH_4 need to be available for thousands of individual cows. Over the past few decades, several methods have been developed to directly measure CH_4 emissions from individual cows (de Haas et al., 2017; Negussie et al., 2017; Zhao et al., 2020). Respiration chambers (RC) are generally considered as the gold standard to measure emissions from individual animals (Hammond et al., 2016a). A RC is an airtight chamber that is usually designed to house a single animal, where all incoming and outgoing gases are continuously measured. By measuring the airflow within the RC, a direct measurement of the total CH_4 emitted by the animal can be obtained. However, measuring individual animals in a RC is time-consuming, labour-intensive, and expensive, making it unsuitable for phenotyping large numbers of individual cows. In addition, RC only provide short-term measurements while the cow is in the RC, which is generally for an hour to a few days, which does not provide information on CH_4 emissions during the full production cycle of the cow. Furthermore, separating the cow from its day-to-day environment, and housing it in a RC, may change feeding behaviour which could potentially influence CH_4 emissions (Hammond et al., 2016a). To overcome some of the challenges, alternative methods have been developed that can be operated long-term and on a larger scale, maximizing the number of animals that can be phenotyped. Nonetheless, each of the methods has its advantages and

disadvantages for large scale application (Garnsworthy et al., 2019). The methods relevant to this thesis will be highlighted in the following paragraphs.

In this thesis, the main focus lies on phenotyping using infrared spectroscopy sensors named 'sniffers' (Figure 1.4). Sniffers are used to measure CH_4 and CO_2 concentrations from the breath of cattle, and are often placed near an AMS, with a tube leading from the feed bin of the AMS to the sniffer that is pumping air from the feed bin (Madsen et al., 2010; Teye et al., 2009). Thereby, the breath concentrations of an individual cow are measured, while the cow is eating the pellets provided in the feed bin of the AMS during milking. The devices are relatively inexpensive compared to other techniques to measure CH_4 and can easily be integrated with existing facilities, such as AMS, where a large number of cows can be measured during the day (Garnsworthy et al., 2019). A disadvantage of sniffers is that only short, so called, "spot-samples" can be recorded during each time that the cow is visiting the AMS. However, long term recording of spot-samples is possible, as long as the cow is lactating.

Figure 1.4. The sniffers as used for phenotyping methane emissions for this thesis. A cow standing in an automated milking system (AMS), where breath is sampled from the feed bin, the sniffer is installed near the AMS at the top left (*left*). The sniffer records a value of 215 ppm methane and 2,795 ppm carbon dioxide (*right*)

Another disadvantage of sniffers is that because of the low airflow of sniffers, not all emissions from the cow will be captured, and thus the total CH_4 production cannot be measured (Huhtanen et al., 2015; Wu et al., 2018). However, methods have been developed to extrapolate short term CH_4 concentration methods to total emissions in g/day. For example, total CH_4 production can be approximated using recovery rates (Garnsworthy et

al., 2012a) or by using predicted CO_2 production as a tracer gas (based on e.g. body weight, milk production, and feed intake) (Madsen et al., 2010). On the other hand, also without knowing the total CH_4 production in g/day, the CH_4 concentration measurements could potentially be used to rank cows from low to high emitters, which would be sufficient for breeding purposes (Garnsworthy et al., 2019). Next to the low capture rate, sniffer measurements are faced with inaccuracies due to environmental conditions, such as: wind and draft going through the AMS, movement of the cows, and the design and volume of the feed bin of the AMS. Statistical modelling and taking repeated measurement over a longer period of time and averaging these measurements is expected to leverage some of the measurement errors (Falconer and Mackay, 1996). Nonetheless, repeated recording and modelling cannot resolve all measurement error and some inaccuracy will remain (Wu et al., 2018). Due to the lower accuracy, sniffers have potential to serve as an indicator for total CH_4 production, however, even with suitable modelling and inference of the data, it is challenging to form conclusions on the true CH_4 production of cows. Therefore, ideally newly developed sniffer sensors are always benchmarked to more reliable methods that can measure individual cows' CH_4 production.

A piece of equipment to measure CH_4 that is similar to sniffers is the GreenFeed system (GF; C-lock Inc. Rapid City, SD, US; Zimmerman (2011)). The GF is a standalone unit, that provides a cow concentrate as bait, and measures CH_4 and CO_2 concentrations while the cow is eating the concentrate during spot-sample visits that generally last a few minutes, several times a day. The GF registers when the cow has its head in the feed bin, and measurements taken while the cows' head is not in the feed bin are discarded. Furthermore, similar to RC, the GF uses an air flux method with a strong pump, to be able to capture all emissions from breath and extrapolate the measurements to total emissions in g/day (Huhtanen et al., 2015). Compared to sniffer systems, the GF is more accurate but also more costly. The costs limit its use for phenotyping full production cycles on thousands of individual cows for breeding programmes (Garnsworthy et al., 2019). However, since the GF system has a higher throughput than RCs, its measurements are potentially valuable for benchmarking other direct or indirect methods of measuring CH_4 emissions from individual animals, such as sniffers.

1.2.2 Agreement between methods

What sniffers and GreenFeed units have in common is that both methods give an estimate derived from a spot-sample of CH_4 emissions from breath while the cow is eating concentrate. The methods are not able to precisely measure the total animal emissions during a full day or longer. Therefore, the agreement not only between measurements from the two methods, but also between measurements of each method and the true total CH_4 production in g/day is of interest. The total CH_4 production can be measured in RC, where we assume

that the CH_4 production recorded for an individual cow in a RC reflects the cows' total production within the herd on-farm.

In the literature, several studies have estimated phenotypic correlations between different methods to measure CH_4 emissions, including RC, sniffers and GF units. For GF units, high phenotypic correlations with RC measurements have been reported of 0.85 and 0.96 (0.96 is transformed from R squared (R^2)) (Hristov et al., 2018; Velazco et al., 2016). For sniffers, several studies report correlation estimates with RC measurements, however, most of the comparisons are difficult to interpret. Interpretation is made difficult because of differences in trait definitions (e.g. derived from peaks in CH_4 concentrations, Garnsworthy et al. (2012a)), random measurement noise in single samples, or because sniffers are placed directly in a RC, and these measurements do not correspond to on-farm conditions as was shown by Difford (2018). One study reported correlations between CH_4 concentration measured by on-farm sniffers and CH_4 production measured in RCs and reported a moderate phenotypic correlation of 0.34 ± 0.22 and a high individual level correlation of 0.75 ± 0.20 (Difford et al., 2019). In addition, one study reported a moderate Pearson's correlation between sniffer and GF measurements (0.30, transformed from R^2 (Huhtanen et al., 2015)). All aspects considered, measurements by the GF system appear to be highly correlated to RC measurements, whereas estimates on the relationship between sniffers and RC have been associated with a larger uncertainty. For sniffers, the type of sniffer, recording set-up, the number of records that are averaged, and trait definition, can have a large influence on the agreement between methods. Therefore, the relationship between sniffer phenotypes and CH_4 production (from e.g. RC or GF), and especially the genetic relationship, should ideally be re-evaluated. The genetic relationship provides important information about the accuracy when using sniffer records to predict GF records, and especially the genetic progress that can be expected in mitigating CH_4 production when selecting for phenotypes derived from sniffer measurements.

1.3 Implementation of Methane in Breeding Programmes

1.3.1 Trait definition

Before a new trait or phenotype can be implemented in a breeding programme, the trait should be clearly defined, recordable, affordable, have phenotypic variation, be heritable, and the genetic correlations with other selection index traits needs to be known (Hazel, 1943). Worldwide, research groups and breeding organisations are working on including methane mitigation in breeding programmes. Often, different methods of phenotyping are used, with each their own defined traits and units of measurement. For example, for research that has investigated the use of sniffer for genetic parameter estimations some studies that measured

CH_4 with sniffers have analysed average CH_4 concentrations (Garnsworthy et al., 2019; Manzanilla-Pech et al., 2022a; Pszczola et al., 2017), whereas other studies defined a phenotype from peaks in CH_4 concentrations (Bell et al., 2019; Garnsworthy et al., 2012a). Because of these differences, it is difficult to compare results and to combine data across countries and for the highly variable sniffer concentration measures, it will be challenging to quantify the realised on-farm or national reduction in emissions.

Currently there is no consensus on which direct CH_4 traits are the most promising for breeding programmes that aim to mitigate CH_4 emissions. To answer these questions, more research will be required that aims to standardise traits, or as highlighted above, additional estimates of phenotypic or genetic correlations can provide valuable knowledge into the relationships between different CH_4 recording methods and different phenotypes defined from one recording method. In addition, for the estimation of breeding values the number of records and duration of CH_4 recording with sniffers is important. Longer recording periods give higher accuracies initially, but plateaus at a maximum accuracy, and due to cost a long recording period may not always be feasible. Furthermore, the genetic variance has been shown to vary during the lactation indicating that the moment of recording is also of importance (Manzanilla-Pech et al., 2022a; Pszczola et al., 2017). When new traits are developed, the optimal phenotyping strategy for that trait should be considered and further investigated. Reflecting on different phenotyping strategies gives important insight into the expected genetic gain, and it helps to apply phenotyping instruments in a cost-effective and time-efficient manner.

1.3.2 Heritability

Several studies have estimated heritabilities for different CH_4 phenotypes in cattle, where the estimates in dairy cattle ranged from 0 to 0.53 ± 0.06 (Table 1.1). The average heritability of the estimates reported in the table is 0.19 and the estimates provide confidence that there is a genetic component to enteric CH_4 emissions, and thus that genetic selection for CH_4 mitigation is possible. However, the large differences between estimates also stresses that it is important to consider the phenotyping method, trait definition, and model choice for breeding value estimations. For example, some studies analysed an average of all CH_4 records, whereas other studies used a repeatability model including averages per AMS visit, day, or week. Deviations in parameter estimates can results in achieving lower genetic progress than what was estimated using a different model or trait. Thus, it is important to re-estimate genetic parameters when making changes in phenotyping practices, trait definition, or when a new and largely unrelated population of animals is recorded, as has been done with sniffers in the Netherlands (Falconer and Mackay, 1996).

1.3.3 Genetic correlations with other breeding goal traits

When selecting for reduced CH_4 emissions, it is important to know the genetic relationships between CH_4 emissions and other breeding goal traits. The current selection index for the Dutch national breeding goal for dairy cattle includes 15 traits from the following categories: milk production, milk components, feed efficiency, health, reproduction, longevity, and conformation (CRV, 2023). Relationships between traits can be estimated as genetic correlations, which are essential parameters to ensure that selecting for reduced CH_4 emissions does not negatively impact other traits in the breeding goal. To date, few genetic correlations have been estimated between CH_4 emissions and current breeding goal traits. For the estimates that are published, they are often associated with large standard errors probably due to a limited numbers of records (Hossein-Zadeh, 2022). Thus, the effect of genetic selection for lower CH_4 emissions on other breeding goal traits is still largely unknown, whereas they are required to make informed breeding decisions. Additionally, genetic correlations could be informative in deciding if other traits can be used as predictor traits for cows without CH_4 records (Negussie et al., 2017). Using predictor traits is useful when the accuracy of the estimated breeding value (EBV) for the predictor trait, multiplied by the genetic correlation, is higher than the accuracy for the EBV for the recorded CH_4 trait.

Table 1.1. Summary of heritability (h^2 ± SE) estimates for methane emissions in dairy cattle, recorded by GreenFeed units (GF), Laser Methane Detectors (LMD), the SF_6 tracer gas technique (SF_6), and sniffers (transformed to production in g/day or L/day, as concentrations in ppm)

Method of recording	Number of cows	Number of records	Mean ± SD	h^2 ± SE	Reference
Combined dataset[1] (g/day)	2,990	15,320	392 ± 86	0.21 ± 0.04	Manzanilla-Pech et al. (2021)
GF (g/day)	330	1,765	464 ± 115	0.16 ± 0.10	Kamalanathan et al. (2023)
GF (g/day)	451	451	492 ± 81	0.36 ± 0.12	
LMD (mg/kg)	57	173	180 ± 47	0.05 ± 0.07	Pickering et al. (2015)
SF_6 (g/day)	205		111 ± 34	0.23 ± 0.23	Manzanilla-Pech et al. (2016)
SF_6 (g/day)	379	1,712	469 ± 81	0.16 ± 0.11	Richardson et al. (2021b)
Sniffer (g/day)[2]	184	2,456		0.12 ± 0.16 to 0.45 ± 0.11	Breider et al. (2019)
Sniffer (g/day)	339		395 ± 58	0.24 ± 0.15	Lassen et al. (2016)
Sniffer (g/day)[2,3]	425 & 318	8,065 & 5,801	307 ± 78 & 355 ± 82	0.11 ± 0.07 to 0.47 ± 0.13	Manzanilla-Pech et al. (2022a)
Sniffer (g/day)[2]	483	34,429	279 ± 64	0.23 ± 0.12 to 0.3 ± 0.08	Pszczola et al. (2017)
Sniffer (g/day)[2]	483	34,359	396 ± 60 & 503 ± 93	0.13 to 0.26	Sypniewski et al. (2021)
Sniffer (g/day)	575	19,126	338 ± 86	0.21 ± 0.05	Manzanilla-Pech et al. (2022c)
Sniffer (g/day)	1,501		183 ± 67	0.12 ± 0.04	Lopez-Paredes et al. (2020)
Sniffer (g/day)	1,745		315 ± 36	0.21 ± 0.06	Lassen and Lovendahl (2016)
Sniffer (g/day)	1,844	5,554	355 ± 73	0.12 ± 0.03	Manzanilla-Pech et al. (2022b)

Sniffer (L/d)	1,397	1,397	380 ± 60	0.25 ± 0.07	Zetouni et al. (2018)
Sniffer (ppm)	337		853 ± 278	0.12 ± 0.01	Saborío-Montero et al. (2019)
Sniffer (ppm)	434	25,724		0.26 ± 0.11	Difford et al. (2020)
Sniffer (ppm)	483	34,359	511 ± 178	0 to 0.14	Sypniewski et al. (2021)
Sniffer (ppm)[2,3]	489 & 368	11,243 & 8,405	576 ± 54 & 573 ± 51	0.10 ± 0.06 to 0.53 ± 0.06	Manzanilla-Pech et al. (2022a)
Sniffer (ppm)	647	26,664	574 ± 52	0.20 ± 0.05	Manzanilla-Pech et al. (2022c)
Sniffer (ppm)	656	16,341		0.15 ± 0.15	Difford et al. (2020)
Sniffer (ppm)	1,508	123,369	254 ± 230	0.11 ± 0.02	van Engelen et al. (2018)
Sniffer (ppm)	1,501		1,288 ± 468	0.11 ± 0.03	Lopez-Paredes et al. (2020)
Sniffer (ppm)	1,962	7,227	577 ± 46	0.15 ± 0.03	Manzanilla-Pech et al. (2022b)

[1]Including measurements from GF, SF_6, and sniffers, [2]Using a random regression model over a lactation, [3]For lactation one and two separately

1.4 Aims and Outline of this Thesis

The main objective of this thesis was to estimate genetic parameters for CH_4 emissions to form the basis for implementing CH_4 mitigation strategies in the Dutch national breeding programme for dairy cows. Data analyses were performed on a previously collected dataset with CH_4 emissions recorded on 15 commercial dairy farms in the Netherlands and for this thesis this dataset was extended with CH_4 emissions recorded on an additional 57 commercial dairy farms. Together, this formed the largest dataset of long-term recorded enteric emissions phenotypes of dairy cows to date.

To reach the main objective, several research aims were formulated. The first aim of this thesis was to estimate the heritability and repeatability of several traits defined from CH_4 concentrations measured by sniffers, and to evaluate the accuracy of breeding values for different CH_4 traits and recording strategies with varying numbers of records and recorded daughters per sire (**Chapter 2**). Subsequently, an additional dataset with CH_4 recorded by GF units was used. From the combined GF and sniffer data, the repeatability and heritability of CH_4 and CO_2 production recorded by GF were estimated and the genetic correlation between CH_4 concentration recorded with sniffers and CH_4 production recorded with GF units (**Chapter 3**). Including the newly recorded sniffer CH_4 measurements on the additional farms, variance components over a lactation were estimated, and used to describe the effect of the moment of recording CH_4 in a lactation on the accuracy of breeding values (**Chapter 4**). Thereafter, genetic correlations were estimated between CH_4 concentration and the main production traits: milk production, protein yield, protein percentage, fat yield, and fat percentage, and body weight and dry matter intake. These traits were chosen, as they were expected to have moderate to high genetic correlations with CH_4 emissions and are economically of importance (**Chapter 5**).

In the general discussion (**Chapter 6**), a synthesis of the results of this thesis is presented along with future perspectives for implementing CH_4 mitigation in breeding programmes. Additionally, the lessons learned in recording CH_4 emissions of dairy cows with sniffers are reflected upon, providing valuable insights for those interested in recording individual CH_4 emissions of cattle for animal breeding purposes.

2

Genetic parameters for repeatedly recorded enteric methane concentrations of dairy cows

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Published in Journal of Dairy Science, Volume 105, Issue 5, Pages 4256-4271, May 2022

DOI https://doi.org/10.3168/jds.2021-21420

Abstract

Animal breeding techniques offer potential to reduce enteric emissions of ruminants to lower the environmental impact of dairy farming. The aim of this study was to estimate the heritability and repeatability of methane (CH_4) concentrations, using the largest dataset from long-term repeatedly recorded CH_4 on cows to date, and to evaluate (1) the accuracy of breeding values for different CH_4 traits, including using visits or weekly means, and (2) recording strategies (with varying numbers of records and recorded daughters per sire). The data comprised of long-term recording of CH_4 and carbon dioxide (CO_2), from 1,746 Holstein Friesian cows, on 14 commercial dairy farms throughout the Netherlands. Emissions were recorded in 10- to 35-s intervals, between 64 and 436 d, depending on farms. From each robot visit, CH_4 and CO_2 concentrations were summarised into various traits, averaged per visit and per week: mean, median, mean log, and mean CH_4/ CO_2 ratio. Genetic parameters were estimated with animal repeatability models, using a restricted maximum likelihood procedure, and a relationship matrix based on genotypes and pedigree. The heritability was equal for mean and median CH_4 per visit (0.13) but lower for logCH_4 and CH_4/CO_2 (0.07 and 0.01, respectively). Phenotypic and genetic correlations were high (≥0.78) between the CH_4 traits, apart from the genetic correlations with the CH_4/CO_2 trait, which were negative. To achieve a minimum reliability of 50% for the estimated breeding value of a bull, 25 records on mean CH_4, measured on 10 different daughters, were sufficient. Although the heritability and repeatability were higher for weekly (0.32 and 0.68, respectively) than for visit mean CH_4 (0.13 and 0.30, respectively), the reliabilities of estimated breeding values from visit or weekly means were equal; thus, we found no advantage in averaging records to weekly means for genetic evaluations.

Key words: methane emissions, genetics, dairy cows

2.1 Introduction

Ruminants produce methane (CH_4) by anaerobic fermentation in the rumen, which is emitted in the air mostly through breathing and belching (Herrero et al., 2016). Reducing these emissions can help to lower the environmental impact of dairy farming. Although enteric CH_4 emissions have been hypothesised to be carbon neutral (Mitloehner et al., 2020), mitigation still contributes to reducing the total emissions of the sector. Animal breeding offers an opportunity to achieve a permanent, cost-effective, and cumulative reduction in enteric CH_4 emissions, which can be implemented in addition to changes in nutrition and manure management (Knapp et al., 2014). To apply breeding techniques, large-scale recording of individual enteric CH_4 emissions is essential (de Haas et al., 2017).

Large-scale and cost-effective recording of individual cows is possible by using "sniffers" (Garnsworthy et al., 2019; Madsen et al., 2010). Sniffers use infrared spectroscopy to measure gas concentrations from the breath and belching of cows. The devices are installed in the feed bin of automated milking stations (AMS), where continuous measurements of the CH_4 concentration (ppm) in the air are taken. Sniffers do not use an air flux measurement, and therefore they cannot measure the CH_4 production in grams per cow per day. However, studies have shown high correlations (0.75 ± 0.20 and 0.89 ± 0.07) between on-farm sniffer measurements in parts per million and respiration chamber measurements, in which the exact CH4 emission of an individual cow was measured (Difford et al., 2019; Garnsworthy et al., 2019). This suggested potential in using sniffers to quantify the variation in enteric CH_4 emissions between cows, and that the measurements could be used to rank cows from low to high emitters for animal breeding practices.

From CH_4 concentrations measured by sniffers, genetic parameters for several traits have previously been estimated. Many studies have used averages of measured CH_4 concentrations (ppm), for which the heritability ranged between 0.11 ± 0.02 and 0.26 ± 0.11 (Difford et al., 2020; Saborío-Montero et al., 2019; van Engelen et al., 2018). Other studies have predicted CH_4 production (g/d) from CH_4 concentrations (ppm) by using CO_2 as tracer gas, combined with the CH_4/CO_2 ratio, as described in Madsen et al. (2010). The heritability for this predicted CH_4 production ranged between 0.12 ± 0.16 and 0.45 ± 0.11 (Breider et al., 2019; Difford, 2018; Lassen and Lovendahl, 2016; Pszczola et al., 2017; Zetouni et al., 2018). Some studies have discussed CH_4 traits in relation to other relevant breeding goal traits, such as milk production (CH_4 intensity), DMI (CH_4 yield), or residual CH_4 (de Haas et al., 2017). Of those 3 traits, only CH_4 intensity has a published heritability for dairy cows, estimated as 0.21 ± 0.06 (Lassen and Lovendahl, 2016). Trait definition affects estimates of heritabilities and genetic correlations, although in the literature differences between estimated genetic parameters have also been large when the same trait definition was used. Large differences in parameter estimates might also be due to different recording strategies and circumstances

between studies. Furthermore, many currently published estimates are associated with large uncertainties shown by high standard errors. Most initial studies have either a small number of records per cow, a small number of recorded cows, or a small number of recorded farms. However, accurate estimates are needed to derive the expected accuracy of breeding values for different trait definitions and recording strategies, important aspects of setting up a breeding programme (Falconer and Mackay, 1996).

In the Netherlands, a breeding goal will be developed to reduce enteric CH_4 emissions of dairy cows, for which phenotypes are being collected in AMS by sniffers. The aim of this study was to estimate the heritability and repeatability of CH_4 concentrations, using the largest dataset from long-term repeatedly recorded cows to date, and to use the data and the corresponding estimates to evaluate (1) the accuracy of breeding values for different traits (and visits or weekly means) and (2) recording strategies (with varying number of records and recorded daughters per sire). The heritability and genetic correlation of alternative CH_4 and CO_2 traits were also estimated. Data were available for continuous and repeated recording of CH_4 and CO_2 concentrations by sniffers between 64 and 436 d, on 14 commercial dairy farms throughout the Netherlands, with a total of 1,746 dairy cows. The heritabilities and genetic correlations between different CH_4 traits and the described recording strategies provide tools to aid discussions that are needed to construct new breeding goals aiming to reduce enteric CH_4 emissions of dairy cows.

2.2 Materials and Methods

2.2.1 Data

Enteric CH_4 emissions were recorded in AMS on 14 commercial dairy farms located throughout the Netherlands, between March 2019 and September 2020. On these farms, a total of 475,555 AMS visits from 2,414 dairy cows were recorded. Emissions were recorded by sniffers (WD-WUR version 1.0, Carltech BV). On each farm, a unique device was installed near the AMS, with an air inlet leading from the feed bin of the AMS. Various types of AMS systems were used in the study, manufactured by Lely Industries NV, GEA Group, DeLaval BV, and Fullwood Packo BV. On each farm, at most 1 AMS was equipped with a sniffer, even if multiple AMS were used within a herd. Before installation in the AMS, sniffers were calibrated using flacons of CH_4, CO_2, and nitrogen. The sniffers could measure CH_4 concentrations in a range of 0 to 2,000 ppm and CO_2 concentrations in a range of 0 to 10,000 ppm.

On each farm, air was sampled continuously, and every 10 to 35 s (varying between devices) a mean was uploaded to the cloud using Arduinos (SODAQ SARA SFF R410M developer board). A data check was performed twice a week to ensure that the sniffers had no sudden change in the mean or variation in emissions. Genotype data, pedigree data, and other

phenotypic data were made available by the cooperative cattle improvement organization CRV (Arnhem, the Netherlands). Animals were genotyped with the Eurogenomics 10K chip. The genotype data comprised 1,817 animals with 76,438 SNPs (imputation was routinely performed by CRV), of which 1,611 cows were phenotyped for CH_4 emissions. The additional 206 cows that were genotyped but not phenotyped were included to maintain linkage between the small number of herds and the large number of bulls with few daughters. Phenotypic data provided by CRV included test-day milk yield, breed composition, and calving dates.

2.2.2 Matching Records to Cow Identification Numbers

Sniffers are not able to record cow identification numbers. Therefore, to match a sniffer measurement with an identification number, the CH_4 measurements were aligned with identification numbers recorded by the AMS. The records could not be merged based merely on timestamps, because the times of sniffers were set manually, whereby inaccuracies may have occurred, and changes to and from daylight saving time were not registered. Therefore, we used an algorithm that matches the sniffer and AMS records, based on the AMS entry time and peaks of CH_4 emissions in the sniffer data, which were located with the function "findpeaks" from the package "pracma" in R version 3.6.1 (Borchers, 2019; R, 2019). This method is similar to that described by Garnsworthy et al. (2012a). A peak was detected based on an increase of 500 ppm of CH_4, with at least 3 increasing datapoints before the peak, followed by 3 decreasing datapoints. A match was defined as a peak that occurred within 30 s before or after the AMS entry time. A 30-s timeframe around the AMS entry was necessary (±time), because the AMS only recorded entry time to the minute, whereas sniffers recorded time at the level of seconds. The time difference with the most matches between sniffer CH_4 peaks and AMS entry times was considered to be the true time difference. Time differences were confirmed by visual inspection before matching the dataset. When data were not matched correctly, many milkings would not result in increases of CH_4, whereas for correctly matched data it could clearly be observed that CH_4 concentrations peaked at the start of milkings and would be low and stable when the robot was empty

2.2.3 Data Editing

The continuous CH_4 measurements within an AMS visit were used to define various CH_4 and CO_2 traits, of which details are described subsequently in the section "Methane Traits." Milking robot visits that did not result in a milking were discarded, as these cows would not receive pellets and would therefore not put the nose in the feed bin close to the air inlet for a longer period of time. Within AMS visits, records taken during the first minute of milking were discarded. This was to ensure that the cow had reached the feed bin and to account for a delay in the air sample entering the air inlet and reaching the sensor. The CH_4 recording period was defined to last at least 2.5 min, to capture not only breathing but also the belching

of cows (van Soest, 1994). Last, records after 5 min of milking were discarded, because after 6 min a decrease in the mean CH_4 emission of all visits combined was observed. This is most likely because, on average, after the first 6 min of milking, cows have finished eating the pellets and would move the nose away from the air inlet, resulting in a decrease of mean emissions.

Background emissions were filtered by subtracting the lowest 1% quantile of records during milking on the day of the measurement from all individual CH_4 and CO_2 measurements of that day. Records from cows up to 305 DIM were analysed, to correctly match the CH_4 records to calving dates and the corresponding parity. Cows whose breeds were less than 75% Holstein were removed from the dataset. The final dataset comprised 308,968 visits from 1,746 individual cows, which all had pedigree data, and 1,611 of these cows also had genotype information.

2.2.4 Methane Traits

For each AMS visit, a mean, a median, a mean of the log of CH_4 and CO_2 emissions, and a mean CH_4/CO_2 ratio were estimated from the concentrations (ppm) measured every 10 to 35 s (Table 2.1). Furthermore, a second dataset was created, from which a mean per week trait was calculated from all visit traits. The mean per week trait included only weeks with a minimum of 7 robot visits per cow recorded. The dataset with weekly means comprised 17,320 records on 1,579 cows. The traits (mean, median, log, and CH_4/CO_2 ratio) were selected based on what has been used previously in the literature (Difford et al., 2016; Lassen and Lovendahl, 2016; van Engelen et al., 2018), which typically include one or two of these traits but never all four as a mean per visit and per week. Traits based on predictions of CH_4 production were not included in the analyses. Recently it has been shown that the method commonly used to predict CH_4 production from CH_4 and CO_2 concentrations (as described by Madsen et al. (2010), is likely to favour inefficient cows over efficient cows in ranking them from low to high CH_4 emitting (Huhtanen et al., 2020). This is most likely a result of biased estimates of CO_2 production, due to differences between cows in their efficiency of energy utilization for maintenance or milk production. Additionally, CH_4 traits defined as a ratio to other relevant breeding goal traits, such as milk production (CH_4 intensity) and DMI (CH_4 yield), or as a residual CH_4 trait, were not included in the analyses, because information on milk yield and DMI were not available.

Table 2.1. The mean (± standard deviation (SD)), minimum, and maximum of the traits defined for enteric methane (CH_4) and carbon dioxide (CO_2) emissions as visit means (308,968 records on 1,746 cows) or weekly means (17,320 records on 1,579 cows)

	Visit emissions			Weekly emissions		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Mean CH_4 (ppm)	328 ± 269	0	1,999	367 ± 216	1	1,587
Median CH_4 (ppm)	315 ± 278	0	2,000	357 ± 221	1	1,856
Mean log CH_4	2.1 ± 0.9	-1.3	3.3	2.2 ± 0.6	-0.3	3.1
Mean CO_2 (ppm)	3,802 ± 1,981	3	9,692	3,820 ± 1,660	143	9,239
Median CO_2 (ppm)	3,853 ± 2,131	2	9,804	3,867 ± 1,762	140	9,505
Mean log CO_2	3.5 ± 0.4	0.3	4.0	3.4 ± 0.3	2.1	4.0
Mean CH_4/CO_2 ratio	0.10 ± 0.11	0	1	0.13 ± 0.12	0	1

2.2.5 Genetic Parameter Estimation

Genetic parameters were estimated with univariate animal models, which included repeated records, using the restricted maximum likelihood (REML) procedure in ASReml 4.2 (Gilmour et al., 2015). From the pedigree and genotype data, a combined genetic relationship matrix ($\mathbf{H}^{-1}$ matrix) was constructed following the method of Aguilar et al. (2010) and Christensen and Lund (2010), using calc_grm version r1.143. Before constructing the $\mathbf{H}^{-1}$ matrix, the pedigree was pruned to only include phenotyped animals and their ancestors, using the statistical programming software R v3.6.1 and the R-package "optiSel" (Wellmann, 2020). The pruned pedigree comprised of 34,394 animals, of which 1,746 were phenotyped for CH_4 emissions. The final $\mathbf{H}^{-1}$ matrix comprised of all 34,394 pedigreed animals.

The significance of fixed effects on the defined CH_4 traits were analysed in ASReml. The random effects included were the additive genetic, parity by permanent environmental, and residual effect. The following model was defined:

$$y_{ijklmno} = \mu + HYW_i + Farm_j.Hour_k + Dur + \sum_{l=0}^{3} DIM_l\,\beta_l + Par_m + a_n + Parity_o.PE_n + e_{ijklnmo}$$

Where y is the phenotype for a CH_4 trait; μ is the mean; HYW is the fixed effect of herd*year*week where the measurement was taken (i = 1 to 1,120); Farm.Hour is the fixed interaction between farm (j = 1 to 14) and hour of the day (k = 1 to 24) and was only used on

the visit data; Dur is the fixed effect for duration of the visit and was only used on the visit data (excluded for weekly); DIM is the fixed regression coefficient for days in milk; β_l is the term of the 3rd order Legendre polynomial for days in milk; Par is the fixed effect of parity (m = 1 to 4, where 4 is parity 4 or higher); a is the random additive genetic effect of the nth animal, $a_n \sim N(0,\mathbf{H}\sigma^2_a)$, where **H** is the combined relationship matrix, and σ^2_a is the additive genetic variance; Parity.PE is the permanent environmental effect of the cows per parity (o = 1 to 11), $\text{Parity}_o.\text{PE}_n \sim N(0,\mathbf{I}\ \sigma^2_{pe})$, where **I** is the incidence matrix and σ^2_{pe} is the permanent environmental variance per parity; and e is the residual error, $e_{ijklnmo} \sim N(0,\mathbf{I}.\ \sigma^2_e)$, where σ^2_e is the error variance.

To estimate phenotypic and genetic correlations between the various CH_4 and CO_2 traits, a series of bivariate analyses were carried out. The same animal model as described previously, including covariances between the residual, genetic, and permanent environmental effects, was used to carry out the analysis for each pair of traits.

From the variance estimates, the heritability and repeatability of the various CH_4 and CO_2 traits were estimated (Mrode, 2005). The heritability was defined as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

The repeatability was defined as:

$$t = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

Where σ^2_a is the additive genetic variance, σ^2_{pe} is the permanent environmental variance, and σ^2_e is the error variance.

2.2.6 Reliability of Estimated Breeding Values

Two types of reliabilities were compared in this study. The model reliabilities of estimated breeding values (EBVs) were obtained directly from the model above, and were defined as (Gilmour et al., 2015):

$$r^2 = 1 - \frac{SE^2}{\sigma_a^2}$$

Where SE is the ASReml standard error of the EBV, and σ^2_a is the estimated additive genetic variance.

These model reliabilities, derived directly from the data, take into account the true relationships that exist between all animals. Furthermore, predicted reliabilities of EBVs were derived from quantitative genetics theory, based on the number of repeated records or

the number of daughters per sire (Table 2.2; Mrode, 2005). These reliabilities assume there are only records available for an individual cow or records are only available on half sib daughters of a bull, and no other relationships exist in the data. The predicted reliabilities for phenotyped cows with repeated records were calculated as:

$$r_{cow}^2 = \frac{n\, h^2}{1 + (n-1)\, t}$$

Where n is the number of records, h^2 is the heritability, and t is the repeatability.

Table 2.2. The number of bulls that has one to 130 daughters in the dataset, in classes of ten

Number of daughters	Number of bulls
1-10	382
11-20	18
21-30	5
31-40	4
41-50	1
61-70	2
121-130	1

The predicted reliabilities for sires of phenotyped cows with a predefined number of daughters were then calculated as:

$$r_{sire}^2 = \frac{\frac{1}{4}\, d\, r_{cow}^2}{1 + \frac{1}{4}(d-1) r_{cow}^2}$$

Where d is the number of daughters, and r^2_{cow} is the derived reliability for the breeding value of the phenotyped daughters, i.e. equivalent to h^2 when only one visit or weekly record is available per cow.

2.3 Results

2.3.1 Average Methane Emissions

The overall mean CH_4 emission per visit was 367 ppm, with a standard deviation of 216 ppm (CV = 59%; Table 2.1, Appendix Figures A1 and A2). The number of days with records ranged from 64 to 436 d for different farms, and depended on the moment of installation and downtime due to maintenance on the sniffers. Mean CH_4 and CO_2 emissions varied between farms and ranged from 99 to 562 ppm, and from 1,161 to 5,186 ppm, respectively (Table 2.3). On average, the mean CH_4 emissions peaked at the start of the lactation, after which the emissions gradually decreased, and so the CH_4 emissions follow the standard lactation curve of milk yield (Figure 2.1). By visual inspection of the phenotypic data, diurnal patters were observed on most farms. In general, the mean CH_4 emissions were observed to decline during the night, increase in the morning, and stay high throughout the day. On four farms, a diurnal pattern could not be observed, for which the CH_4 emissions remained constant throughout the day.

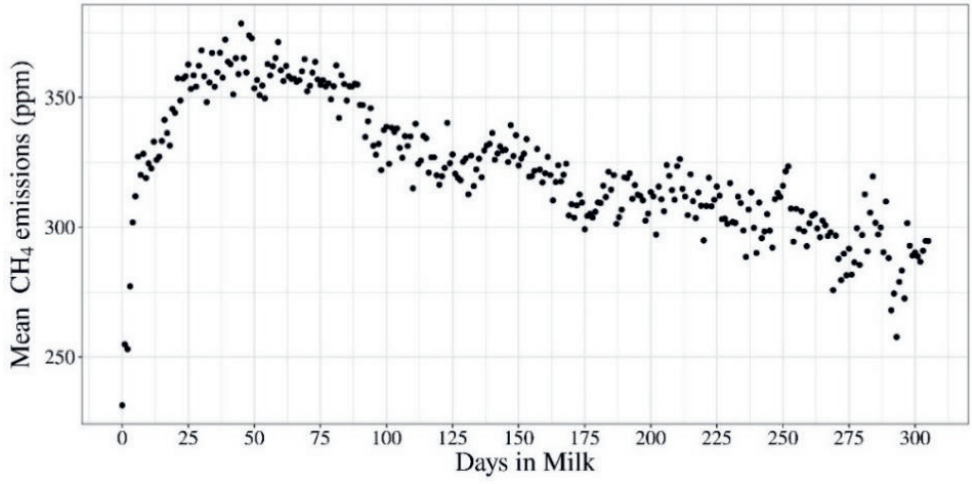


Figure 2.1. The mean methane (CH_4) emissions (ppm) per AMS visit on each day in milk

Table 2.3. Descriptive statistics per farm for the number of cows, the total number of days with records (there can be gaps in the recording period), the total number of visits recorded, the mean methane (CH_4) (ppm) and carbon dioxide (CO_2) (ppm) emissions (± standard deviation (SD))

Farm	No. cows	No. days of recording	No. of visits	Mean per visit (ppm)	
				CH_4 ± SD	CO_2 ± SD
1	19	339	2,357	133 ± 151	2,480 ± 1,180
2	118	245	16,978	314 ± 210	3,399 ± 2,198
3	181	261	31,348	99 ± 140	2,289 ± 1,172
4	111	225	18,397	562 ± 315	5,186 ± 1,821
5	193	316	36,598	501 ± 284	4,527 ± 2,255
6	27	64	756	536 ± 248	1,161 ± 598
7	93	347	28,916	279 ± 197	4,025 ± 1,196
8	156	177	21,132	348 ± 295	5,039 ± 2,266
9	293	436	60,508	421 ± 257	3,355 ± 2,002
10	188	319	16,234	347 ± 225	4,079 ± 1,320
11	40	104	6,843	150 ± 156	4,601 ± 1,709
12	98	276	31,019	139 ± 168	4,117 ± 1,275
13	145	115	10,885	507 ± 237	5,417 ± 1,878
14	84	413	26,997	220 ± 103	2,491 ± 1,217

2.3.2 Genetic Parameter Estimation

The estimated heritability and repeatability from mean visit emissions were 0.13 ± 0.01 and 0.30 ± 0.01, respectively (Table 2.4). After averaging the records per week, the heritability and repeatability for mean CH_4 significantly increased for all traits and became moderate (0.32 ± 0.03 and 0.68 ± 0.01, respectively; Table 2.4). Averaging the recorded visits per week, resulted in a large decrease of the residual variance (Table 2.5). Mean and median CH_4 emissions had the highest heritability and repeatability. The trait log-transformed CH_4 emissions had a lower heritability than the mean CH_4 emissions (0.23 ± 0.03 for weekly logCH_4 emissions). The ratio trait had the lowest heritability and a low repeatability (0.02 ± 0.01 and 0.15 ± 0.01, respectively, for weekly CH_4/CO_2 emissions).

Table 2.4. The heritability (h^2) and repeatability (t) for the mean methane (CH_4) emissions, median CH_4 emissions, log CH_4 emissions, mean carbon dioxide (CO_2) emissions, median CO_2 emissions, log CO_2 emissions, and CH_4/CO_2 ratio averaged per visit and week (± SE)

	Visit		Week	
	h^2	t	h^2	t
mean CH_4 (ppm)	0.13 ± 0.01	0.30 ± 0.01	0.32 ± 0.03	0.68 ± 0.01
median CH_4 (ppm)	0.13 ± 0.01	0.29 ± 0.01	0.32 ± 0.03	0.68 ± 0.01
logCH_4	0.09 ± 0.01	0.18 ± 0.01	0.23 ± 0.03	0.65 ± 0.01
mean CO_2 (ppm)	0.16 ± 0.02	0.36 ± 0.01	0.33 ± 0.03	0.71 ± 0.01
median CO_2 (ppm)	0.16 ± 0.01	0.35 ± 0.01	0.34 ± 0.03	0.71 ± 0.01
logCO_2	0.07 ± 0.01	0.20 ± 0.01	0.20 ± 0.03	0.57 ± 0.01
CH_4/CO_2	0.01 ± <0.01	0.08 ± <0.01	0.02 ± 0.01	0.15 ± 0.01

2.3.3 Genetic Correlations Between Methane Traits

Phenotypic and genetic correlations were estimated between the weekly emission traits. The estimated genetic correlations between the mean, median, and logCH_4 traits were high (0.78–1.00; Table 2.6). On the contrary, the phenotypic and genetic correlations between the CH_4/CO_2 ratio trait and all other traits were negative (−0.08 to −0.45 and −0.27 to −0.99, respectively), and the genetic correlations had high standard errors (0.16–0.29). Furthermore, the phenotypic correlations between CH_4 and CO_2 emission traits were high (0.70–0.85).

Table 2.5. The genetic (σ^2_a), permanent environmental (σ^2_{pe}), error (σ^2_e), and phenotypic variance (σ^2_p) of mean methane (CH_4) and carbon dioxide (CO_2) emissions per robot visit and per week (± SE)

	σ^2_a	σ^2_{pe}	σ^2_e	σ^2_p
Visit CH_4	5,308 ± 543	6,429 ± 335	28,024 ± 72	39,760 ± 457
Weekly CH_4	5,371 ± 610	6,115 ± 388	5,381 ± 62	16,867 ± 493
Visit CO_2	411,526 ± 42,557	505,494 ± 25,976	1,607,350 ± 4,111	2,524,400 ± 35,279
Weekly CO_2	416,517 ± 47,117	471,873 ± 29,585	357,251 ± 4,149	1,245,600 ± 37,896

2.3.4 Reliability of Estimated Breeding Values

Predicted reliabilities for EBV were estimated based on the heritability and repeatability estimates for mean visit and weekly emissions. The predicted reliability for EBV of phenotyped cows with repeated records increased steeply for the first 10 weekly records of mean CH_4 emissions, after which the gain per additional recording per cow became smaller (Figure 2.2). For visits, the increase in reliability was slightly less steep, and the gain per additional recording became smaller after around 25 recorded visits. For phenotyped cows, one weekly mean gave a higher reliability than one visit, which is a result of the higher heritability for weekly means (Figure 2.2 and Table 2.7). However, when considering an average of 7 visits per week, the two reliabilities were approximately equal (7 visits per week versus 1 weekly). This was calculated from the parameter estimates and applying a sensitivity analysis based on their standard errors. The predicted reliability of EBV for sires increased when a larger number of daughters were recorded per sire, and the level of reliability depended on the number of records for mean CH_4 emission per daughter (Figure 2.3). With a larger number of records per daughter, the reliability of sires was higher, although after 10 recorded weeks or 25 recorded visits, the gain in adding extra records became negligible. For sires with a large number of recorded daughters that all have a large number of records, the difference between the reliability for visit emissions and weekly emissions decreased (Table 2.7). For cows with own performance information, including repeated records, the realised reliability for mean CH_4 emission was higher than that expected based on the predicted reliability (Figure 2.4 A). For sires, the realised reliability, which included all records, followed the expected pattern from predicted reliabilities (Figure 2.4 B). Figure 2.4 A confirms that the largest increase in reliability came from the inclusion of approximately the first 25 repeated records. By including more repeated records, the reliability for cows only marginally increased.

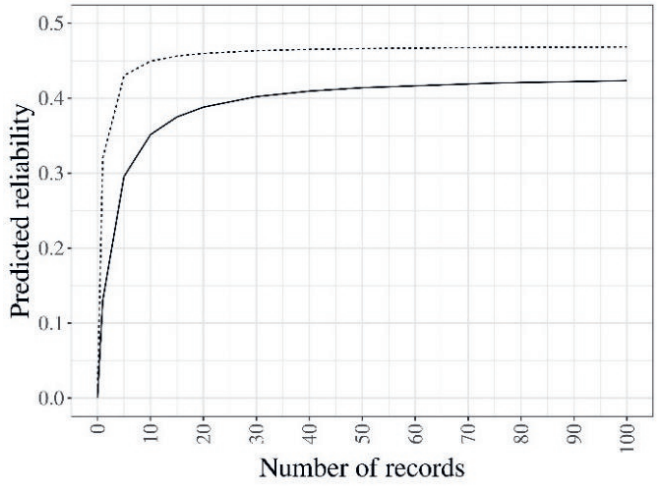


Figure 2.2. The predicted reliability for the mean CH_4 emission for cows with own performance information, based on the number of phenotypic records per cow. Calculated for the number of recorded visits (Solid), and the number of recorded weeks (Dashed). The lines were derived from the corresponding estimates of heritability and repeatability (resp. 0.13 and 0.30 for visits, and 0.32 and 0.68 for weeks)

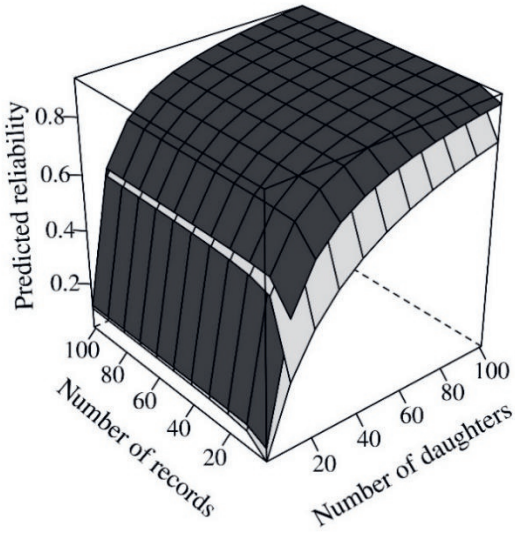


Figure 2.3. The predicted reliability for sires with phenotyped daughters, based on the number of records on mean CH_4 emission per daughter and the number of daughters per sire. The reliability was derived for both visit means (Light grey) and weekly means (Dark grey) from the corresponding estimates of heritability and repeatability (resp. 0.13 and 0.30 for visits, and 0.32 and 0.68 for weeks)

Table 2.6. Phenotypic and genetic correlations between weekly methane (CH_4) and carbon dioxide (CO_2) traits. The phenotypic correlations are reported above the diagonal, the genetic correlations below the diagonal (± SE)

	Mean CH_4 (ppm)	Median CH_4 (ppm)	LogCH_4	Mean CO_2 (ppm)	Median CO_2 (ppm)	LogCO_2	CH_4/CO_2
Mean CH_4 (ppm)		0.99 ± <0.01	0.72 ± 0.01	0.85 ± <0.01	0.84 ± <0.01	0.72 ± 0.01	-0.09 ± 0.01
Median CH_4 (ppm)	1.00 ± <0.01		0.70 ± 0.01	0.83 ± 0.01	0.83 ± 0.01	0.70 ± 0.01	-0.08 ± 0.01
LogCH_4	0.80 ± 0.02	0.78 ± 0.02		0.71 ± 0.01	0.70 ± 0.01	0.70 ± 0.01	-0.09 ± 0.01
Mean CO_2 (ppm)	0.90 ± 0.01	0.88 ± 0.01	0.76 ± 0.02		0.99 ± <0.01	0.88 ± <0.01	-0.22 ± 0.01
Median CO_2 (ppm)	0.89 ± 0.01	0.88 ± 0.01	0.75 ± 0.02	1.00 ± <0.01		0.87 ± <0.01	-0.21 ± 0.01
LogCO_2	0.89 ± 0.02	0.88 ± 0.02	0.80 ± 0.02	0.99 ± 0.01	0.99 ± 0.01		-0.45 ± 0.01
CH_4/CO_2	-0.56 ± 0.22	-0.55 ± 0.22	-0.27 ± 0.16	-0.99 ± 0.30	-0.95 ± 0.29	0 ± 0	

Table 2.7. The predicted reliabilities for cows with own performance information and sires with phenotyped daughters. The reliabilities for cows are derived from the number of visit or weekly records, and the heritability and repeatability for visit or weekly means. The reliabilities for sires are derived from the cows' reliability from n records, and the number of daughters

Number of records per cow	Predicted reliability cow		Number of daughters per sire	Predicted reliability sire	
	Visit	Weekly		Visit	Weekly
1	0.13	0.32	5	0.14	0.30
			10	0.25	0.47
			100	0.77	0.90
5	0.30	0.43	5	0.29	0.38
			10	0.44	0.55
			100	0.89	0.92
10	0.35	0.45	5	0.33	0.39
			10	0.49	0.56
			100	0.91	0.93
25	0.40	0.46	5	0.35	0.39
			10	0.52	0.57
			100	0.92	0.93
50	0.41	0.47	5	0.37	0.40
			10	0.54	0.57
			100	0.92	0.93
100	0.42	0.47	5	0.37	0.40
			10	0.54	0.57
			100	0.92	0.93

2.4 Discussion

The aim of this study was to estimate the heritability and repeatability of CH_4 concentrations, using the largest dataset from long-term repeatedly recorded cows to date, and to use the data and corresponding estimates to evaluate (1) the accuracy of breeding values for different traits (visits or weekly means) and (2) recording strategies (with varying numbers of records and recorded daughters per sire). Data were available on continuous and repeated recording of CH_4 and CO_2 emissions, from 1,746 dairy cows, on 14 commercial dairy farms throughout the Netherlands. From the data, genetic parameters were estimated with univariate and

bivariate animal models, using a restricted maximum likelihood procedure. The results show that mean CH_4 emissions had moderate heritability and repeatability, but that there was no advantage in averaging the mean emissions per week to estimate breeding values for sires. From the mean emissions per visit, 25 records measured on 10 different daughters gave reliabilities of breeding values above the Dutch publication threshold of 50%.

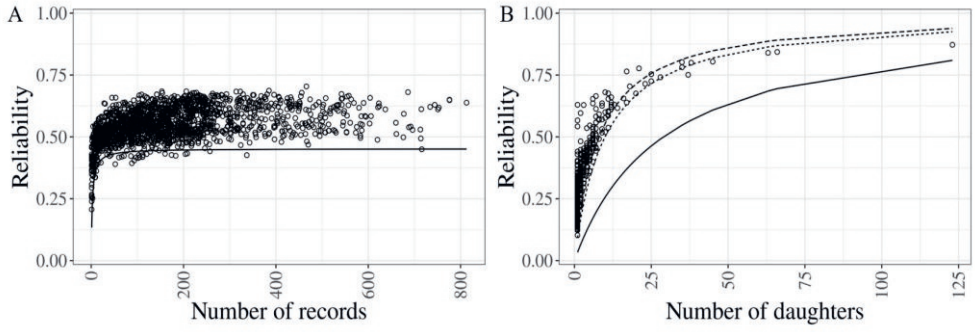


Figure 2.4. The realised reliability of estimated breeding values (EBVs) (dots) and expected predicted reliability (line) for cows with own performance information on the mean CH_4 emissions per robot visit, by their number of recorded visits (A), and for sires of phenotyped cows by their number of daughters, which assumes each cow has 1 (solid line), 10 (small dashed line), or 100 (large dashed line) repeated records (B)

2.4.1 Phenotypic Analysis

The mean CH_4 emissions recorded in this study was 367 ppm, which is within the low range of previously estimated means from similar sniffer devices (between 254 and 1,288 ppm; van Engelen et al., 2018; Bell et al., 2019; Difford et al., 2019; López-Paredes et al., 2020; Saborío-Montero et al., 2020). Low records can be a result of variations in measurement conditions and drifting of sensors toward zero. This may also have caused the coefficient of variation (CV) to increase to higher levels than expected and caused differences in the CV between farms. By correcting for differences between farms and between measuring weeks within farms in the genetic analysis, we assume that these factors have minimal influence on the estimates of the genetic parameters. Another concern that is often raised is that CH_4 measurements by sniffers are inaccurate or biased and are influenced by systematic environmental effects, random errors, and systematic errors (Huhtanen and Hristov, 2018). Systematic environmental effects, such as conditions during the day or farm, are not problematic for genetic analysis and can be separated from genetic effects by the separation of environmental effects in mixed model analysis. Similarly, random errors, which can be a result of movement of the cow's nose and the position of the nose in the feed bin (Wu et al., 2018), do not have to be problematic and can be reduced by taking multiple repeated

measurements of each cow. Furthermore, the effect of the position of the nose and head lifting was expected to be reduced by only considering the first five minutes of milking, where we expect most cows were eating concentrates. Systematic errors, such as behaviour during milking, are more serious if the errors are also partly genetic in nature. For example, cows that are restless in nature might look around in the milking robot more frequently, causing the measured concentrations to decline. It should be further studied whether this behaviour is repeatable and heritable, as has also been pointed out by Wu et al. (2018). Nonetheless, even if sniffer measurements party reflect differences in other traits, CH_4 measured by sniffers could still serve as in indicator for true CH_4 emissions (Bovenhuis et al., 2018).

The pattern of CH_4 emissions over DIM (Figure 2.1) and the diurnal pattern indicate that sniffers are able to detect variation in emissions. Diurnal patterns have been reported previously (Bell et al., 2014c; Pszczola et al., 2017; van Engelen et al., 2018), and in these studies the CH_4 emissions increased during the day and decreased during the night, which is similar to patterns observed in this study. A study by Crompton et al. (2011), showed that these diurnal patterns in CH_4 emissions can be caused by changes in feed intake during the day. In our study, the relationship between CH_4 emissions and feed intake was not further investigated, as data on feed intake was not available.

For DIM, earlier studies have reported a steep increase of CH_4 emissions in the first weeks of lactation, with emissions remaining stable or gradually decreasing thereafter. This is in agreement with the pattern for DIM observed in this study (Figure 2.1). Also, feed intake may play a role; daily DMI is typically lower in early lactation compared with mid and late lactation (Krattenmacher et al., 2019).

2.4.2 Methane Traits

In this study we have analysed average CH_4 concentrations (ppm) measured in the feed bin of milking robots. High individual-level correlations have been reported (0.75 ± 0.20) between sniffer CH_4 breath concentration (ppm) measurements and respiration chamber CH_4 production (g/d) measurements; this was published by a study that installed sniffers in milking robots for 3 weeks of lactation and subsequently in respiration chambers (Difford et al., 2019). Therefore, we assumed that analysing concentrations measured in parts per million gives sufficient information about relative differences between cows, which is required to select the best-performing animals for breeding practices. Additionally, we analysed CH_4/CO_2 as a ratio trait that is generally used in quantifying CH_4 production (Madsen et al., 2010). Nonetheless, to gain confidence in using CH_4 concentration measurements from sniffers for genetic evaluations, the relationship with measurements of emissions in grams per day, as is done with the GreenFeed (C-Lock Inc.; Hammond et al., 2016), should be investigated further. This is needed to confirm that the total emissions in grams per day will be reduced by breeding for reduced CH_4 concentrations measured in parts per million.

In the literature, CH_4 measurements in parts per million are often converted to grams per day to approximate a cow's total emission. To predict emissions in grams per day from concentration measurements by sniffers, CO_2 emissions are used as a tracer gas through a formula that assumes a constant efficiency of energy utilization for different metabolic functions (Madsen et al., 2010). This assumption is not always met and can result in CH_4 emissions to be overestimated, on average by 17% for efficient compared with inefficient cows, favouring the inefficient cows (Huhtanen et al., 2020). Other traits that have received interest in the literature are CH_4 intensity (g of CH_4/kg of milk), CH_4 yield (g of CH_4/kg of DMI), and residual CH_4 (observed CH_4 minus predicted, from, e.g. milk yield, CH_4; de Haas et al., 2017). These traits would account for the highly conserved relationships between CH_4, milk yield, and feed intake, and thus rank cows from low to high emitting, regardless of their level of production or feed intake. However, these relationships can also be addressed by including correlation structures between CH_4, milk yield, and feed intake in the selection index. In a simulation study, Zetouni et al. (2017) showed that a multitrait approach resulted in higher genetic gain than by selecting for ratio traits. Additionally, responses to selection for a multitrait index, compared with including a ratio trait, will be easier to interpret, which makes the index more approachable for farmers. Because the interest was in analysing traits that would be suitable to add to a breeding goal, we did not include analyses on CH_4 as a ratio to other breeding goal traits, although more research is needed to verify the effect of using a ratio trait for CH_4 in breeding goals.

Many traits had minimum records of 0 ppm CH_4 (rounded to zero), which is below what is biologically expected (Table 2.1, Appendix Figures A1 and A2). The low records were most likely a result of drifting of the sensor calibration that occurred during the study, where the sensor calibration drifted toward zero. As a result, the CH_4 data were not normally distributed, and therefore a log-transformation on the CH_4 records was performed. Nonetheless, because CH_4 emissions are expected to be normally distributed by nature and moved toward normality after averaging the records to means per week (Appendix Figure A2), we do not recommend using log-transformations of a CH_4 trait in the breeding objective. Furthermore, log-transformation did not solve non-normality of CH_4 in this dataset. Therefore, we recommend investigating other options to correct for drift of sensors in future studies; for example, by using censored models or multiple imputation, or by standardisation of the data based on the week of the measurement.

Other traits derived from sniffer measurements that are often mentioned in the literature are CH_4 emissions estimated from eructation peak traits. Peak traits were not included in this study, because the recording interval of up to 35 s does not provide sufficiently detailed information. However, estimations of the CH_4 emissions from peaks have been shown to be moderately to highly correlated with the average CH_4 concentration during milking (0.62 and 0.86, on different diets; Bell et al., 2014b). Therefore, peak traits may still be of interest for

breeding when using different recording practices, where concentrations are measured, for example, every second.

2.4.3 Genetic Parameters

The estimated heritabilities were highest for mean and median CH_4 emissions averaged per week (both were 0.32 ± 0.03; Table 2.4). As expected, averaging the recorded visits to records per week resulted in an increased heritability. Earlier studies reported heritabilities for the mean CH_4 concentration (ppm) and mean logCH_4 concentration per week, measured by sniffers, ranging from 0.11 ± 0.03 to 0.26 ± 0.11 (Difford et al., 2020; Lopez-Paredes et al., 2020), and for mean CH_4 production per week of 0.12 ± 0.16 in a repeatability model (Breider et al., 2019), and of 0.25 ± 0.07 from one weekly record (Zetouni et al., 2018). Thus, the heritability estimated for weekly mean emissions in this study is somewhat higher than what has been observed in literature. This could have been a result of the large quantity of data used and the inclusion of genomic information, as well as of the requirement of a minimum number of seven records per weekly mean to come to a more reliable average. The heritability for mean CH_4 concentration per visit (0.13 ± 0.01 for mean CH_4 and 0.09 ± 0.01 for logCH_4) is similar to the heritability reported by van Engelen et al. (2018) for logCH_4 concentration (0.11 ± 0.02) and by Saborío-Montero et al. (2019) for average CH_4 concentration over a period of two to three weeks (0.12 ± 0.01). Estimates that have been reported in the literature for the heritability of mean CH_4 production per visit were somewhat higher, at 0.21 ± 0.06 and 0.19 ± 0.09 (Difford, 2018; Lassen and Lovendahl, 2016). Residual variances may differ between farms; therefore it is of interest to estimate intra-herd variances. However, we were not able to estimate genetic parameters within farms, because most farms had a limited number of cows recorded, with only one farm having recorded over 200 cows (n = 293; Table 2.3).

The repeatabilities estimated in this study for mean and median CH_4 emissions were moderate and, again, higher for weekly than for visit mean emissions (0.68 ± 0.01 and 0.30 ± 0.01, respectively; Table 2.4). Repeatability estimates for CH_4 concentrations recorded by sniffers have been published previously. In the literature, repeatabilities of mean CH_4 concentrations per visit were 0.42 and 0.45 ± 0.07 (Rey et al., 2019; Sypniewski et al., 2019), and 0.27 ± <0.01 and 0.33 for logCH_4 (Difford et al., 2016; van Engelen et al., 2018). Thus, the repeatabilities estimated in this study for mean CH_4 emissions per visit were lower than previously published estimates (0.30 and 0.18 for mean CH_4 and logCH_4, respectively). For CH_4 production per visit or per day, repeatability estimates have been reported that ranged from 0.36 to 0.66 ± 0.11 (Haque et al., 2015; Negussie et al., 2017; Sorg et al., 2018), which is in the same range as, or higher than, the repeatabilities reported for CH_4 concentrations. Estimates of the repeatability for log CH_4 concentrations averaged per week ranged from 0.47 to 0.84 (Difford et al., 2016; Difford et al., 2019; Difford et al., 2020). The repeatability estimated in this study for weekly logCH_4 emissions (0.65 ± 0.01) falls within this range.

In this study, the CH_4/CO_2 ratio trait had a low heritability (0.01 ± <0.01 to 0.02 ± 0.01) and a low repeatability (0.08 ± <0.01 to 0.15 ± 0.01). Low estimates have also been reported in the study by van Engelen et al. (2018), where the heritability was 0.03 ± 0.01 and the repeatability was 0.14 ± <0.01. However, for the CH_4/CO_2 ratio trait, Lassen and Lovendahl (2016) reported a higher estimate of heritability of 0.16 ± 0.04, although in that study emissions were measured for seven days and averaged over the full recording period. A low heritability indicates that a larger number of records per cow is needed for the CH_4/CO_2 ratio trait to accurately estimate EBV, compared with direct measurements of CH_4 concentration, which were shown to be moderately heritable in this study. Using CH_4 in a ratio trait has other disadvantages. First, as has been addressed previously, direct selection on the trait of interest is more advantageous, as it realises a higher genetic response than indirect selection on a ratio trait (Zetouni et al., 2017). Second, interpretation would be more difficult, as the level of feed intake, efficiency of energy utilization, and body energy balance can also influence the gas ratio (Huhtanen et al., 2015). Finally, Huhtanen et al. (2015) suggest that air-mixing conditions, caused by the geometry of the feed bin, might influence the CH_4/ CO_2 ratio.

Genetic correlations between the mean, median, and logCH_4 traits were high (0.78–1.00), indicating that these traits can be used interchangeably after standardisation (Table 2.6). In contrast, the phenotypic and genetic correlations between the CH_4/CO_2 ratio trait and all other traits were negative. Again, this shows that the CH_4/CO_2 ratio is most likely less suitable for application in selection indices and not a good indicator of a cow's CH_4 emission. However, in our study the ratio may have been influenced by the drift of CH_4 sensors while the CO_2 sensor remained stable, making the relationship between the ratio and its component traits nonlinear, and therefore could be more informative when sensors are calibrated regularly.

The phenotypic and genetic correlations between CH_4 and CO_2 concentrations were positive. The phenotypic correlations ranged from 0.70 ± 0.01 to 0.85 ± <0.01, and the genetic correlations ranged from 0.75 ± 0.02 to 0.90 ± 0.01. This is in agreement with what has been reported in an earlier study, where the phenotypic correlations ranged from 0.87 ± 0.01 to 0.96 ± <0.01, and the genetic correlations ranged from 0.96 ± 0.03 to 0.97 ± 0.03 (Difford et al., 2020). Furthermore, a study that used respiration chamber measurements also reported a high phenotypic correlation between CH_4 and CO_2 production (0.93; Aubry and Yan, 2015). The high correlations suggest that a strong relationship exists between the quantity of emitted CH_4 and CO_2.

2.4.4 Recording Strategies

For cows with own performance information, the predicted reliability appears higher when estimated from the mean CH_4 emission per week compared with the reliability estimated from visits, for the same number of records per cow (0.46 and 0.40, for 25 recorded weeks

or visits respectively). However, in this scenario a weekly mean is expected to be calculated from at least seven recorded visits, and, when comparing the reliability from seven visits to one week, no gain in reliability is detectable for either scenario. Therefore, to estimate reliable EBV, we found no gain in averaging the recorded visits per week. Similarly, for sires the reliabilities of EBV were approximately equal for one weekly record versus seven recorded visits. This also illustrates that heritability should always be interpreted in context of the trait definition.

The reliability of EBV estimated from own performance information is constrained by the large difference between the heritability and the repeatability, which is the permanent environmental effect (Falconer and Mackay, 1996). This effect can also be observed in Figure 2.4 A, where the predicted reliabilities, derived from quantitative genetics theory, are plotted based on the number of repeated measurements. By taking measurements further apart in time, the permanent environmental variance could possibly be reduced. When the time gap between records increases, the environmental correlation between records may decay faster than the genetic correlation, which could result in a higher reliability for cows. Although this effect was not investigated in this study, it might be useful in optimizing recording strategies and for efficient use of recording equipment, but should be investigated further. Given that CH_4 emissions have been shown to have a different genetic background over DIM (Pszczola et al., 2017), it is important to consider lactation stages. Nonetheless, for a large number of animals, the realised reliabilities by ASReml were higher expected based on the predicted reliabilities (Figure 2.4). The higher reliability can be explained by the inclusion of extra information on relatives. The predicted reliabilities assume that only CH_4 records on the individual animal are available, whereas in the data daughters, half-sibs and full sibs may also have phenotypic records, which provide additional information about the individual animal.

From Figure 2.4 B, we can derive the number of daughters that should be phenotyped in practice to be able to reliably estimate breeding values for the mean CH_4 emissions for bulls. The Dutch cattle improvement organization CRV publishes breeding values for milk production traits when the reliability is above 50%, and for all other traits when the reliability is above 25% (CRV, 2020). Using these numbers, in Figure 2.4 we can observe that around 10 daughters per sire, with at least 10 visits recorded on mean CH_4 emissions per daughter, would need to be recorded to reach an accuracy of 50% for sires. However, only a handful of daughters, with at least 10 repeated records per daughter, would need to be recorded to achieve a reliability of 25%. These numbers of records are determined by the estimated heritability and repeatability and assume that these are estimated without error. However, in our study the standard errors (SE) were between <0.01 and 0.04, and therefore a sensitivity analysis was performed, which assumed that the heritability or the reliability (or both) were

± 2 SE. This analysis showed that the required minimum number of records did not change within the expected range of error of the heritability and repeatability estimates.

From the results, we suggest that at least 25 visits should be recorded on cows to accurately calculate EBV for mean visit CH_4 emissions for the phenotyped cows or their sires in a repeatability model (Figures 2.2 and 2.3). A study using the GreenFeed system, showing the computation of a cow's CH_4 or CO_2 production rate, required a minimum of 30 recorded visits, each lasting more than three minutes, to obtain a reliable average of multiple short-term breath measurements (Arthur et al., 2017). This indicates that, despite the GreenFeed system's more accurate recording, by correcting the measurements for areal conditions and movement of the head of the cow (Huhtanen et al., 2015), the minimum recording period of the system is similar to the minimum recording period of the sniffer. Additionally, sniffers have the advantage of the ability to record a larger number of cows per recording period, equal to the capacity of the AMS, which is a prerequisite for genetic evaluations for which hundreds to thousands of animals need to be phenotyped. Nonetheless, it is important to realise that these recommendations relate to mean CH_4 concentrations with heritability and repeatability in the magnitude of what has been estimated in this study. When new traits are defined, with different parameter estimates, or different models are applied, the recording strategies have to be re-evaluated.

2.4.5 Implications

This study confirms that there is promise in using CH_4 emissions measured by sniffers in genetic evaluations. Mean CH_4 emissions (ppm) per visit and per week have moderate to high heritability and repeatability, are easy to record and easy to interpret as absolute differences between cows, and could most likely serve as an indicator for total CH_4 emissions (g/d), as is suggested by the high correlations between sniffer and respiration chamber measurements (Difford et al., 2019; Garnsworthy et al., 2019). Methane emissions are currently not included in dairy selection indices around the world. Large-scale phenotyping is required first, to investigate the relationships between CH_4 emissions and other selection index traits. However, currently not enough measurements were available to derive the relationships between CH_4 emissions and other breeding goal traits, which are required before inclusion in a selection index.

It has to be further investigated which trait or traits defined from CH_4 emissions measured by sniffers should be added to a breeding goal that aims to reduce enteric CH_4 emissions per animal. Previous studies have shown that the heritability of CH_4 emissions changes over a lactation, and that the genetic correlation between different DIM was on average 0.74 and could decrease to 0 for DIM further apart (5 vs. 305 DIM; Pszczola et al., 2017; Breider et al., 2019; Sypniewski et al., 2021). This shows that it is important to consider the period in which CH_4 was recorded, and not simply to assume a genetic correlation of one between

different DIM, as was done with the repeatability model in this study. Random regression models have the advantage of allowing for heterogeneous genetic and residual variances between lactation stages, and can model underlying genetic correlations, similar to models that are used for milk yield in the Netherlands (CRV, 2018). Additionally, correction for heterogeneity of variances might be required to adjust for different variances across herds. Better modelling of the underlying genetic structure will most likely improve the reliability of breeding value estimations and reduce the need for recording in different lactation periods. Random regression models will be investigated with this dataset when more data has been collected. Nonetheless, the current estimates of heritability clearly indicate that, also by using a simpler repeatability model, genetic progress can be made.

2.5 Conclusions

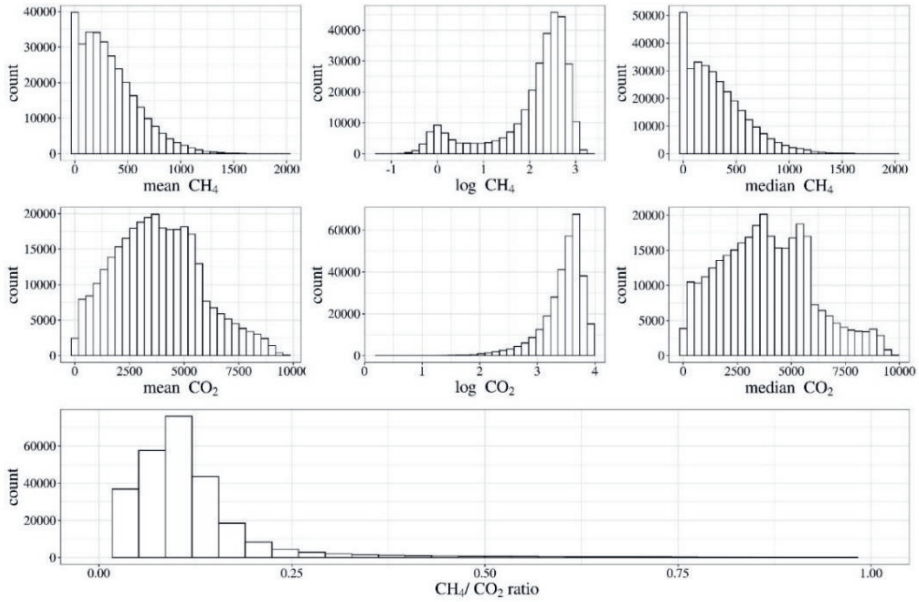
In this study, genetic parameters were estimated for CH_4 concentrations continuously measured in the feed bin of milking robots. Moderate heritability and repeatability were estimated for mean and median CH_4 emissions. Low heritability was estimated for the ratio trait of CH_4/CO_2. Phenotypic and genetic correlations were high between the mean, median, and logCH_4 traits, excluding the CH_4/CO_2 ratio trait, which was negative. From the mean CH_4 emissions per visit, 25 records on mean CH_4, measured on 10 different daughters, gave reliabilities of breeding values above the Dutch breeding value publication threshold of 50%. Although the heritability and repeatability for the mean emissions per week were higher than for the mean emissions per robot visit, the reliabilities of estimated breeding values derived from the two recording strategies are equal.

Acknowledgements

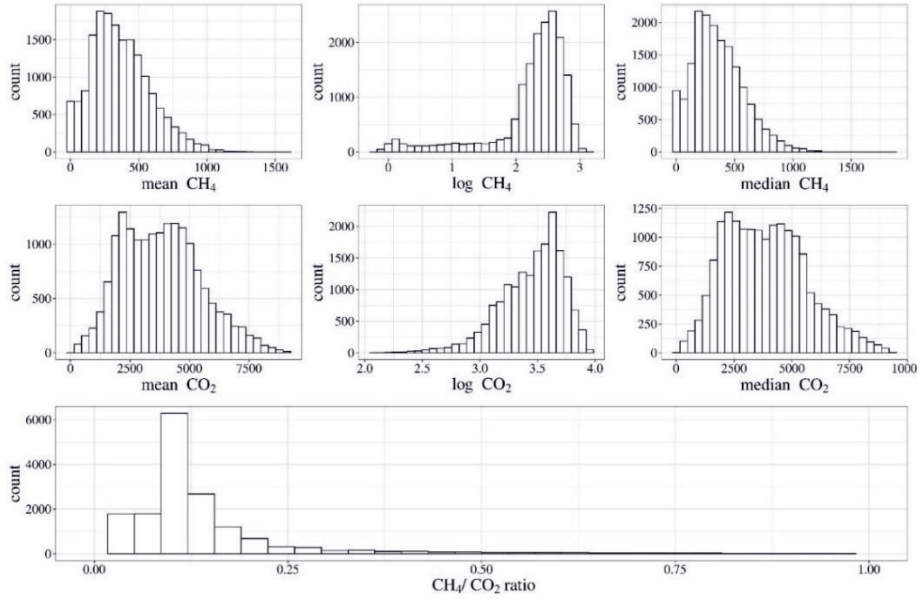
The research leading to these results was part of the Climate Approach Project, funded by the Dutch Ministry for Agriculture, Nature and Food Quality (LNV, The Hague; project number BO-53-003). This work was supported by the TKI Agri & Food project LWV19155 (Wageningen, the Netherlands) and the partners CRV (Arnhem, the Netherlands) and FrieslandCampina (Amersfoort, the Netherlands). The authors gratefully acknowledge the farmers for participating in this research, Johan Ploegaert (Wageningen Livestock Research, Wageningen, the Netherlands) for maintaining the sniffers, Hendrik Jan van Dooren (Wageningen Livestock Research) for setting up data collection, and CRV for providing the pedigree and genotype data. The authors have not stated any conflicts of interest.

Appendices

A1. Histograms for the methane traits per robot visit: mean CH_4, log CH_4, median CH_4, mean CO_2, log CO_2, median CO_2 and the CH_4/CO_2 ratio



A2. Histograms for the methane traits as an average per week: mean CH_4, log CH_4, median CH_4, mean CO_2, log CO_2, median CO_2 and the CH_4/CO_2 ratio



3

Heritability and genetic correlations between enteric methane production and concentration recorded by GreenFeed and sniffers on dairy cows

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Published in Journal of Dairy Science. Volume 106, Issue 6, Pages 4121-4132, June 2023

DOI https://doi.org/10.3168/jds.2022-22735

Abstract

To reduce methane (CH_4) emissions of dairy cows by animal breeding, CH_4 measurements have to be recorded on thousands of individual cows. Currently, several techniques are used to phenotype cows for CH_4, differing in costs and applicability. However, there is uncertainty about the agreement between techniques. To judge the similarity and repeatability between measurements of different recording techniques, the repeatability, heritability, and genetic correlation are useful metrics. Therefore, our objective was to estimate (1) the repeatability and heritability for CH_4 and carbon dioxide production recorded by GreenFeed (GF) and for CH_4 and carbon dioxide concentration measured by cost-effective but less accurate sniffers, and (2) the genetic correlation between CH_4 recorded with these two different on farm and high throughput techniques. Data were available from repeated measurements of CH_4 production (grams/day) by GF units and of CH_4 concentration (ppm) by sniffers, recorded on commercial dairy farms in the Netherlands. The final data comprised 24,284 GF daily means from 822 cows, 170,826 sniffer daily means from 1,800 cows, and 1,786 daily means from 75 cows by both GF and sniffer (in the same period). Additionally, CH_4 records were averaged per week. For daily and weekly mean GF CH_4 the heritabilities were 0.19 ± 0.02 and 0.33 ± 0.04, and for daily and weekly mean sniffer CH_4 the heritabilities were similar and were 0.18 ± 0.01 and 0.32 ± 0.02, respectively. Phenotypic correlations between GF CH_4 production and sniffer CH_4 concentration were moderate (0.39 ± 0.03 for daily means and 0.37 ± 0.05 for weekly means). However, genetic correlations were high; 0.71 ± 0.13 for daily means and 0.76 ± 0.15 for weekly means. The high genetic correlation indicates that selection on low CH_4 concentrations (ppm) recorded by the cost-effective sniffer method, will result in reduced CH_4 production (grams/day) as recorded with GF.

Key words: methane emissions, genetics, dairy cows, GreenFeed, sniffer

3.1 Introduction

Cattle and other ruminants contribute to methane (CH_4) that is emitted into the atmosphere, which is a significant driver in global warming (Smith et al., 2014). Various strategies have been suggested to reduce emissions from cattle, such as through advances in gut microbiology, nutrition, improved animal health, and genetic improvement by animal breeding (Hill et al., 2016). To breed for cows that emit less CH_4, a large number of individual cows need to be phenotyped first. Several techniques exist to phenotype cows for enteric CH_4 emissions and each method has its advantages and disadvantages (Hammond et al., 2016a).

Methods installed on farm, such as the GreenFeed (GF; C-lock Inc.; Zimmerman, 2011) and "sniffers" (Madsen et al., 2010; Teye et al., 2009), show promise to be used in large-scale recording of dairy cows. Both techniques repeatedly measure CH_4 and carbon dioxide (CO_2) concentrations from the breath of a cow during measurements that generally last a few minutes, and additionally GF units measure quantitative airflow, which is used to calculate CH_4 and CO_2 production (grams/ day) based on mass flux calculations (Huhtanen et al., 2015). However, GF units are currently prohibitive for large-scale recording, due to purchase and running costs, and the limitation of the number of cows that can be recorded (15 to 25 cows per unit as recommended by C-lock). Sniffer systems, however, are cheaper with low running costs, are high throughput, and only limited to the number of cows that have access to the automated milking systems (AMS) where the sniffer is installed (generally 40 to 70 cows; Garnsworthy et al., 2019).

Estimates for the heritability of CH_4 production measured by GF units have been reported, and ranged from 0.12 ± 0.06 to 0.35 ± 0.19 for daily and 0.22 ± 0.11 to 0.43 ± 0.12 for weekly CH_4 production. Estimates for the heritability of CH_4 concentration (ppm) measured by sniffers were similar, and ranged between 0.11 ± 0.02 and 0.32 ± 0.03 (Difford et al., 2020; Saborío-Montero et al., 2019; van Breukelen et al., 2022; van Engelen et al., 2018). Some studies estimated CH_4 production (grams/day or litres/day) from sniffer concentration measurements, based on CO_2 as a tracer gas in combination with the CH_4/CO_2 ratio (Madsen et al., 2010), or based on an average tidal respiratory volume (Chagunda et al., 2009). For CH_4 production estimated from sniffer measurements, the heritability estimates are again similar and range between 0.12 ± 0.04 and 0.45 ± 0.11 (Breider et al., 2019; Difford, 2018; Lassen and Lovendahl, 2016; Lopez-Paredes et al., 2020; Pszczola et al., 2017; Zetouni et al., 2018).

Not only is the heritability of CH_4 sampling techniques important, but also how measurements of different techniques correlate. Both GF systems and sniffers are spot-sampling methods and do not measure the total "true" emissions per day. Nonetheless, both CH_4 recorded by GF and recorded by sniffers has been shown to be highly correlated with

CH_4 measured in respiration chambers (RC; 0.75–0.96; Velazco et al., 2016; Hristov et al., 2018; Difford et al., 2019). The correlations indicate that measurements by either system are correlated with a cow's total emission as are measured in RCs. However, Huhtanen et al. (2015) investigated the phenotypic correlation between CH_4 recorded by GF and by sniffers, and reported a low correlation of 0.30 (transformed from r^2). Nonetheless, only phenotypic correlations between the CH_4 sampling techniques have been reported in the literature. To be able to judge the similarity between the sampling techniques, the genetic correlation is more useful and can be used as a measure of repeatability between sampling techniques (Veerkamp et al., 2002).

The genetic relationship between CH_4 production recorded by GF and CH_4 concentration recorded by sniffers is important to investigate if selection on low CH_4 concentrations (ppm) recorded by the sniffer method, will result in reduced CH_4 production (grams/day). Therefore, the objective of this study was to estimate (1) the repeatability and heritability for CH_4 and CO_2 production recorded by GreenFeed (GF) units and for CH_4 and CO_2 concentration measured by sniffers, and (2) the genetic correlation between CH_4 recorded by these two different techniques.

3.2 Materials and Methods

3.2.1 Methane Recording

GreenFeed units and sniffers were used to noninvasively record enteric CH_4 and CO_2 emissions from lactating dairy cows on commercial farms in the Netherlands. Ethical approval was not needed for this study because no animal procedures were performed, as only existing data were used. Emissions were recorded with GF units on 16 farms (with 19 to 293 recorded cows per farm) and with sniffers on 15 farms (with 14 to 96 recorded cows per farm). On six of the farms, measurements were taken with both GF units and sniffers. The GF units were used to estimate CH_4 and CO_2 production (CH_4p and CO_2p, respectively) as grams per day in the barn or on pasture, for either two weeks or three months, between September 2018 and February 2020. In total 161,825 visits from 1,184 cows were recorded by GF units. The technical specifications of the GF units had a measurement range for CH_4 concentrations in between 0 and 4,000 ppm, and for CO_2 concentrations between 0 and 20,000 ppm. Full details on the data collection with GF were reported by Koning et al. (2020). Sniffers (WD-WUR v1.0, manufactured by Carltech BV) were installed in the feed bin of AMS and measured CH_4 and CO_2 concentrations (CH_4c and CO_2c, respectively) in ppm, with recording periods from 64 up to 436 days, between March 2019 and January 2021. The concentration measurements were not used to estimate production, because of problems in sensor drift which occurred for CH_4 and CO_2 independently (van Breukelen et al., 2022). In total 461,223 AMS visits from 2,271 cows were recorded by sniffers. The technical specifications of the sniffers had a measurement range for CH_4 concentrations between 0 and

2,000 ppm, and for CO_2 concentrations between of 0 and 10,000 ppm. A detailed description of the data recording by sniffers is given in van Breukelen et al. (2022).

3.2.2 Data Editing

Sniffers do not record cow ID, therefore, the sniffer records were first aligned with ID recorded by the AMS (for more details see van Breukelen et al. (2022)). Thereafter, the GF dataset and sniffer dataset were filtered to only include cows for which pedigree data was available, provided by the cooperative cattle improvement organization CRV (Arnhem, the Netherlands). Furthermore, the datasets were filtered to only include cows that were 75% Holstein or more. Records for cows up to 305 days in milk (DIM) were retained to correctly match the recorded AMS visits to calving dates and the corresponding parity. The data were not Log transformed, as this did not result in normality of the data. Nonetheless, previous analysis on the same data showed that the residuals were normally distributed. A linear model was used to correct both the data recorded by GF units and by sniffers for diurnal variation with a Fourier series approach (Lassen and Lovendahl, 2016; Lovendahl and Bjerring, 2006) using the following model:

$$y_{ik} = \mu + \text{Farm}_i . \sum_{j=1}^{1} (\sin j\theta 2\pi + \cos j\theta 2\pi) + e_{ik}$$

where y_{ik} is GF or sniffer-recorded CH_4 or CO_2 per visit; *Farm* is the fixed effect for the i^{th} farm and is fitted as an interaction with the 24-hour diurnal cycle, where θ is the time at recording as a decimal fraction (i.e., θ = hour at recording / 24), and j is the order of regression; and e is the residual error, $e_{ik} \sim N(0, \mathbf{I}\sigma^2_e)$, where σ^2_e is the error variance. The estimated fixed effects were subtracted from the corresponding records to derive the corrected estimates from each visit.

After correction for diurnal variation, the recorded GF and sniffer visits were combined in one dataset with daily means and one dataset with weekly means. In the dataset with weekly means, records with less than three records per cow per week were discarded. The number of remaining daily and weekly records and cows used for the analyses are summarised in Table 3.1. Recording by GF and sniffers was mostly carried out on different farms, because the GF data were collected for different research objectives in other studies.

Table 3.1. The number of farms, cows, and daily or weekly methane and carbon dioxide records, recorded by GreenFeed (GF), sniffers, or by both methods (in total or with overlapping recording)

Number of	Daily				Weekly			
	GF	Sniffer	GF and sniffer	GF and sniffer overlaps[1]	GF	Sniffer	GF and sniffer	GF and sniffer overlaps[1]
Farms	16	15	6	4	16	15	6	4
Cows	822	1,800	184	75	822	1,800	176	73
Records	24,284	170,826		1,786	4,358	30,982		334

[1] The number of farms, cows, and daily or weekly records for which GF units and sniffers recorded emissions within the same day, note these are a subset of GF and sniffer records

3.2.3 Pedigree and Genomic Data

Pedigree and genotype data were made provided by CRV. In total 1,817 animals were genotyped with the Eurogenomics 10k chip and imputed to 76,439 SNPs by CRV as part of a routine process. The pedigree was pruned to include all phenotyped animals and their ancestors, using the R-packages "optisel" in R v3.6.1 (Wellmann, 2020). In total, the pruned pedigree included 41,290 animals from 29 generations.

3.2.4 Parameter Estimation

Pearson's correlation coefficients were estimated to visually inspect the relationship between CH_4 emissions of 75 cows measured by GF units and by sniffers, without a correction for environmental influences. Fisher's transformation was used to derive the Confidence Intervals (CI) for the transformed Pearson's correlation estimates (Fisher, 1921).

Variance components were estimated with pairwise bivariate repeatability animal models, using a restricted maximum likelihood procedure in ASReml 4.2 (Gilmour et al., 2015). The variance components were used to estimate the heritability, repeatability, and phenotypic, genetic, permanent environmental, and residual correlations. As input, a genetic relationship matrix was used which combines the pedigree and genotype data ($\mathbf{H}^{-1}$). The $\mathbf{H}^{-1}$ matrix was constructed following the method of Aguilar et al. (2010) and Christensen and Lund (2010) using calc_grm version r1.143. The $\mathbf{H}^{-1}$ matrix comprised all 41,290 animals that were in the pedigree.

The statistical significance of fixed effects was tested in ASReml before including the fixed effects in the final model. The random effects included were the additive genetic, permanent environmental, and residual effect. In the bivariate models between a GF and a sniffer trait the permanent environmental covariance was fixed to zero. This was done because the permanent environmental covariances in the analyses between a GF and sniffer trait were not

statistically significant and resulted in spurious estimates, most likely due to the low number of cows that had both records by GF units and by sniffers (Table 3.1). For the bivariate models with two GF or two sniffer traits, the permanent environmental covariances were significantly different from zero and therefore the permanent environmental covariance was not fixed to zero. The bivariate model used in the final analysis was defined as:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1 & 0\\ 0 & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Za}_1 & 0\\ 0 & \mathbf{Za}_2\end{bmatrix}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Zp}_1 & 0\\ 0 & \mathbf{Zp}_2\end{bmatrix}\begin{bmatrix}\mathbf{pe}_1\\ \mathbf{pe}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Ze}_1 & 0\\ 0 & \mathbf{Ze}_2\end{bmatrix}\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

where $\mathbf{y}_i$ is a vector with records on trait *i* (GF CH_4p or CO_2p and sniffer CH_4c or CO_2c, as daily or weekly mean); $\mathbf{b}_i$ is a vector containing fixed effects for trait *i*, which were farm*unit*year*week of the measurement, second breed fraction*second breed, DIM which was modelled using third-order Legendre polynomials, and parity (from parity 1 to 4, where 4 is parity four or higher); $\mathbf{a}_i$ is a vector containing additive genetic effects for trait *i*; $\mathbf{pe}_i$ is a vector containing permanent environmental effects within parity (from parity 1 to 11) for trait *i*; $\mathbf{e}_i$ is a vector with the residuals for trait *i*; and $\mathbf{X}_i$, $\mathbf{Za}_i$, $\mathbf{Zp}_i$, and $\mathbf{Ze}_i$ are identity matrices linking the records in $\mathbf{y}_i$ to the fixed effects, the additive genetic effects, and the permanent environmental effects, respectively. The additive genetic, permanent environmental and residual effects for all traits were assumed normally distributed with a mean of zero, a variance of σ^2_{ji} for random effect *j* and trait *i*, and a covariance between two traits of σ_{j1j2}:

$$\begin{bmatrix}j_1\\ j_2\end{bmatrix} \sim N\left[\binom{0}{0}, \mathbf{I} \otimes \begin{pmatrix}\sigma^2_{j_1} & \sigma_{j_1 j_2}\\ \sigma_{j_1 j_2} & \sigma^2_{j_2}\end{pmatrix}\right]$$

From the variance estimates, heritabilities and repeatabilities were estimated and reported as means of all bivariate runs. The heritability was defined as:

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_e}$$

where σ^2_a is the additive genetic variance, σ^2_{pe} is the permanent environmental variance, and σ^2_e is the residual variance.

The repeatability was defined as:

$$t = \frac{\sigma^2_a + \sigma^2_{pe}}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_e}$$

3.3 Results

The daily mean CH_4p measured by GF units was 436 g/d (Table 3.2) with a coefficient of variation (CV) of 28%. The weekly mean CH_4p was 435 g/d and had a lower CV of 23%. The daily mean CH_4c measured by sniffers was 325 ppm with a high CV of 77%. The weekly

mean CH_4c was 331 ppm with a CV of 66%. The repeatability of CH_4 and CO_2 was higher in the scenarios with weekly means than with daily means, both when measured as production by GF units or as concentrations by sniffers. The repeatability of CH_4p measured by GF units compared with CH_4c measured by sniffers was equal for daily means (0.34), but higher for weekly mean CH_4p than weekly mean CH_4c (0.77 and 0.66, respectively).

Table 3.2. The mean ± standard deviation (SD), minimum, maximum, and repeatability (t) of daily or weekly methane (CH_4) and carbon dioxide (CO_2) emissions, recorded by GreenFeed (GF, g/day) or sniffer (ppm)

		CH_4				CO_2			
		Mean ± SD	Min	Max	t[1]	Mean ± SD	Min	Max	t[1]
GF (g/day)	Daily	436 ± 120	38	1,929	0.34	13,159 ± 2,041	2,590	22,399	0.45
	Weekly	435 ± 98	148	834	0.77	13,169 ± 1,760	7,931	19,971	0.81
Sniffer (ppm)	Daily	325 ± 251	0.3	1,964	0.34	3,725 ± 1,865	3	9,683	0.39
	Weekly	331 ± 218	0.5	1,566	0.66	3,684 ± 1,586	65	9,257	0.69

[1] All repeatabilities had a standard error of 0.01 and were reported as means of all bivariate analyses

Both CH_4p measured by GF units and CH_4c measured by sniffers decreased during the night and were lowest around 06.00h, whereafter the measured CH_4p and CH_4c increased (Figure 3.1). Additionally, both showed a dip around 16.00h. This dip was larger for CH_4c measured by sniffers. Both CH_4p and CH_4c increased rapidly during the first DIM (Figure 3.2). However, after 100 DIM average CH_4c measured by sniffers started to decrease, whereas the average CH_4p measured by GF units was relatively consistent after 100 days.

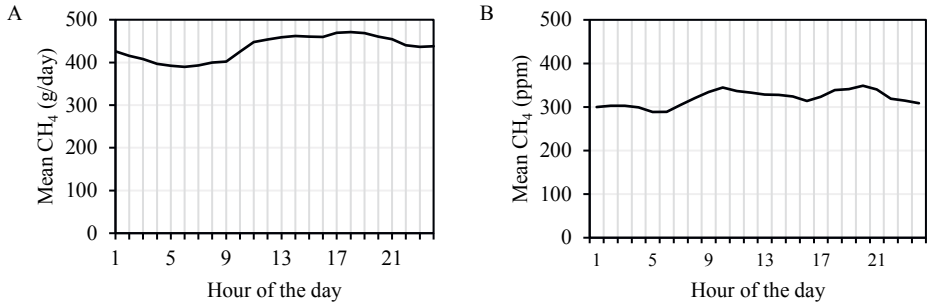

Figure 3.1. The mean CH_4 emissions measured as (A) production (g/day) on 16 farms by GreenFeed units and (B) concentration (ppm) on 15 farms by sniffers per hour of the day

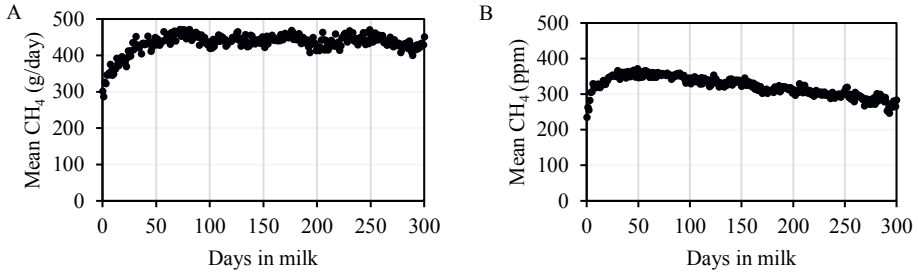

Figure 3.2. The mean CH_4 emissions measured as (A) production (g/day) on 16 farms by GreenFeed units and (B) concentration (ppm) on 15 farms by sniffers per days in milk

In total 75 dairy cows were measured with both GF units and sniffers within the same days (Table 3.1), with on average 24 days with measurements by both techniques. Of these cows, 73 dairy cows had weekly mean measurements, with at least three visits recorded per week, by both techniques within the same week. These 73 cows had on average five weeks of measurements by both GF units and sniffers. The Pearson correlations between GF CH_4p and sniffer CH_4c for these cows were low (0.20 (95% CI [0.15, 0.24]) and 0.19 (95% CI [0.08, 0.29])), for daily and weekly means respectively) (Figure 3.3A). Similarly, the Pearson correlations between GF CO_2p and sniffer CO_2c were low for daily (0.08 (95% CI [0.03, 0.12])), and for weekly means (−0.01 (95% CI [-0.12, 0.0.10])) (Figure 3.3B).

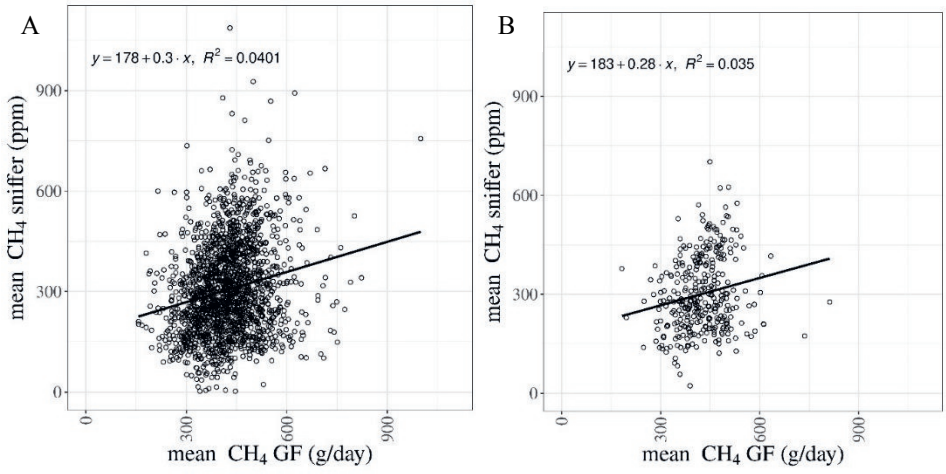

Figure 3.3. The relationship between methane (CH_4) production measured by GreenFeed (GF, g/day) units and CH_4 concentrations measured by sniffers (ppm) from repeated measurements on (A) 75 dairy cows as means per day and (B) 73 dairy cows as means per week

Variance components were estimated with bivariate repeatability animal models and were used to estimate the repeatability, heritability, and phenotypic and genetic correlations. The heritability of CH_4p measured by GF units was 0.20 for daily means and 0.33 for weekly means (Table 3.3). The heritability of CH_4c measured by sniffers was similar; and was 0.18 for daily means and 0.32 for weekly means, albeit the repeatability was slightly lower for the weekly sniffer measures compared with the GF. The phenotypic correlation between daily mean CH_4 measured by GF or sniffer was 0.39 ± 0.03, and 0.37 ± 0.05 between weekly mean CH_4 measured by GF or sniffer. The genetic correlation between daily mean CH_4 measured by GF or sniffer was high (0.71 ± 0.13), and was similar for weekly mean CH_4 measured by GF or sniffer (0.76 ± 0.15). Residual correlations were moderate and ranged from 0.06 ± 0.03 to 0.77 ± <0.01 (Appendix Table A1).

The heritability of CO_2p measured by GF units was 0.24 for daily means and 0.34 for weekly means and the heritability of CO_2c measured by sniffers was 0.20 for daily means and 0.32 for weekly means (Table 3.3). The genetic correlations between CO_2p and CH_4p measured by GF were moderate (0.64 and 0.65, for daily and weekly means, respectively), and were higher between CO_2c and CH_4c recorded by sniffers (0.93 for daily and weekly means). Furthermore, the genetic correlations between CO_2p and CO_2c (0.52 and 0.60, for daily and weekly means, respectively) were lower than the genetic correlations between CH_4p and CH_4c (0.71 and 0.76, for daily and weekly means, respectively).

Table 3.3. Phenotypic (above the diagonal) and genetic correlations (below the diagonal) between methane (CH_4) and carbon dioxide (CO_2) recorded by GreenFeed (GF, production: CH_4p in g/day) units or sniffers (concentration: CH_4c in ppm) and averaged per day or per week (± standard error). The heritabilities are reported on the diagonal [1]

	GF CH_4p day	GF CO_2p day	GF CH_4p week	GF CO_2p week	Sniffer CH_4c day	Sniffer CO_2c day	Sniffer CH_4c week	Sniffer CO_2c week
GF CH_4p day	**0.19 ± 0.02**	0.72 ± 0.01	0.70 ± 0.01 [2]	0.53 ± 0.01	0.39 ± 0.03	0.20 ± 0.04	0.37 ± 0.04	0.18 ± 0.04
GF CO_2p day	0.68 ± 0.04	**0.24 ± 0.03**	0.58 ± 0.01	0.77 ± 0.01 [2]	0.32 ± 0.04	0.25 ± 0.04	0.35 ± 0.04	0.27 ± 0.04
GF CH_4p week	0.99 ± 0.01 [2]	0.66 ± 0.05	**0.33 ± 0.04**	0.75 ± 0.01	0.27 ± 0.04	0.15 ± 0.05	0.37 ± 0.05	0.19 ± 0.06
GF CO_2p week	0.64 ± 0.05	1.00 ± 0.01 [2]	0.65 ± 0.05	**0.34 ± 0.05**	0.22 ± 0.04	0.18 ± 0.04	0.31 ± 0.05	0.24 ± 0.06
Sniffer CH_4c day	0.71 ± 0.13	0.54 ± 0.15	0.74 ± 0.15	0.69 ± 0.16	**0.18 ± 0.01**	0.78 ± >0.01	0.73 ± <0.01 [2]	0.62 ± 0.01
Sniffer CO_2c day	0.39 ± 0.16	0.51 ± 0.15	0.47 ± 0.17	0.63 ± 0.16	0.93 ± 0.01	**0.20 ± 0.01**	0.65 ± 0.01	0.76 ± <0.01 [2]
Sniffer CH_4c week	0.71 ± 0.14	0.60 ± 0.15	0.76 ± 0.15	0.72 ± 0.16	1.00 ± <0.01 [2]	0.92 ± 0.01	**0.32 ± 0.02**	0.84 ± <0.01
Sniffer CO_2c week	0.35 ± 0.17	0.51 ± 0.15	0.41 ± 0.18	0.60 ± 0.17	0.91 ± 0.01	1.00 ± <0.01 [2]	0.93 ± 0.01	**0.32 ± 0.02**

[1] The heritabilities were reported as the mean of all runs

[2] Estimate with the highest likelihood, but with convergence problems due to closeness to unity of the correlation

3.4 Discussion

The aim of this study was to estimate (1) the repeatability and heritability for CH_4 and CO_2 production recorded by GreenFeed (GF) units and for CH_4 and CO_2 concentration measured by sniffers, and (2) the genetic correlation between CH_4 recorded by these two different techniques. In the results we showed that CH_4 and CO_2 emissions recorded by either GF units or sniffers had a moderate heritability and that the genetic correlation between CH_4p measured by GF units and CH_4c measured by sniffers was high.

3.4.1 Heritability and Repeatability

The heritability that we estimated for CH_4p recorded by GF units was moderate, and was 0.19 ± 0.02 for daily means and 0.33 ± 0.04 for weekly means (Table 3.3). The first published estimates of the heritability for CH_4 production measured by GF units ranged from 0.12 ± 0.06 to 0.35 ± 0.19 for daily and 0.22 ± 0.11 to 0.43 ± 0.12 for weekly CH_4 production. In addition, many studies have reported heritability estimates for various traits for CH_4 recorded by sniffers (Lassen and Difford, 2020). Some studies using sniffers attempted to estimate CH_4p from sniffer CH_4c measurements by using mass flux calculations (Madsen et al., 2010) or based on tidal volume (Chagunda et al., 2009). The estimated heritability for GF CH_4p reported in this study, is within the range of the in the literature reported heritabilities for estimated sniffer CH_4p, which ranged between 0.12 ± 0.04 and 0.45 ± 0.11 (Breider et al., 2019; Difford et al., 2018; Lassen and Lovendahl, 2016; Lopez-Paredes et al., 2020; Zetouni et al., 2018).

The repeatability of similar trait definitions for CH_4p measured by GF units and CH_4c measured by sniffers was comparable (0.34 for daily mean CH_4p and CH_4c, Table 3.2). For both sampling techniques, the repeatability was higher when using multiple measurements of weekly mean CH_4, and was 0.77 for CH_4p measured by GF units and 0.66 for CH_4c measured by sniffers. The higher repeatability for weekly means is a result of averaging a larger number of records, which reduces the temporary environmental variance (Falconer and Mackay, 1996). In the literature, many repeatability estimates for CH_4 emissions measured on dairy cows are reported. The literature estimates reported depend largely on trait definition, which is confirmed by the results in this study, where the repeatability estimates are higher for the traits based on weekly mean CH_4 emissions than for daily mean CH_4 emissions. This highlights the importance of carefully defining the trait when reporting parameter estimates.

The literature also reports several repeatability estimates for CH_4p measurements by GF units. The estimates from this study, fall within the range of estimates reported in the literature. For example, Manafiazar et al. (2016) averaged CH_4p measurements in 1 to 14 day means, and estimated repeatabilities ranging from 0.33 to 0.79. Also Coppa et al. (2021)

reported that the repeatability of CH_4p increased when averaging records over longer periods of time (0.60 to 0.78, for one to eight week means). On the contrary, a study by Denninger et al. (2019) analysed 7, 14, and 28 day means (0.64, 0.68, and 0.59, respectively) and showed that the repeatability for CH_4p was highest for 14 day means. Thus, it is uncertain which length of recording period for averaging records yields the highest repeatability. Nonetheless, when measurements are used to estimate breeding values from repeated measurements in a repeatability model averaging visits over longer periods of time, by using weekly means, may increase the heritability and repeatability but will not result in higher reliabilities (van Breukelen et al., 2022).

3.4.2 Genetic and Phenotypic Correlations

For the second objective, we successfully estimated a genetic correlation between CH_4c measurements by sniffers and CH_4p measurements by GF units. This is the first study to estimate a genetic correlation between CH_4 recorded by sniffers and any other CH_4 recording technique in dairy cows. Our results showed that the genetic correlation between CH_4p measured by GF units and CH_4c measured by sniffers was high, and was 0.71 ± 0.13 for daily means and 0.76 ± 0.15 for weekly means (Table 3.3). The genetic correlations between weekly means of CH_4p or CH_4c and daily means of the other trait were similar to the estimates within daily or weekly means (0.71 ± 0.14 and 0.74 ± 0.15).

These high genetic correlations indicate that when cows are selected based on low breeding values for CH_4c measured by sniffers, this would result in reducing the average CH_4p in grams per day as measured by GF units. However, regardless of the large dataset and that the phenotypes are genetically linked, the results of this study were based on a relatively small dataset, with 184 cows recorded by both GF units and sniffers and only 75 cows with records overlapping in time. The permanent environmental covariance was fixed to zero in the bivariate models between a GF and a sniffer trait, because the permanent environmental covariances in these analyses were not significantly different from zero, most likely due to the low number of cows (n = 184) that had both records by GF units and by sniffers (Table 1.1). Furthermore, the low number of records did not allow to fit an across lactation permanent environmental effect, next to the within lactation permanent environmental effect. Therefore, the results should be interpreted with caution and further analyses will be required to gain confidence in these estimates. Nonetheless, the correlation estimates remained similar when the permanent environmental covariance was not fixed to zero, although these analyses did not converge. This does suggest that the reported results are likely to provide a good indication of the expected direction of the correlations, and this study sets the basis of future research on the genetic correlations between different techniques to measure enteric CH_4 emissions.

The phenotypic correlations were moderate between CH_4p measured by GF units and CH_4c measured by sniffers, and were 0.39 ± 0.03 for daily means and 0.37 ± 0.05 for weekly means. The moderate phenotypic correlations suggest that environmental effects segregate the measurements by the two systems. For example, measurements could be affected by differences in the biology and behaviour of the cow (Wu et al., 2018), or are a result of the different samplings techniques used. Nonetheless, the high genetic correlations show that pedigree and genomic information help to link measurements between related individuals, making it possible to disentangle the genetic background of CH_4 emissions from environmental factors. Whereas genetic correlations are missing in the literature, previous studies have investigated phenotypic relationships between CH_4 measurements from different CH_4 recording techniques. As was mentioned in the introduction, it has been shown that both CH_4p measured by GF units and CH_4c measured by sniffers are phenotypically correlated with CH_4p measured in RCs. Studies using GF reported high correlations of 0.85 and 0.96 (0.96 is transformed from r^2; Velazco et al. (2016); Hristov et al. (2018)). A study using sniffers reported a moderate phenotypic correlation of 0.34 ± 0.22 and a high individual level correlation 0.75 ± 0.20 between CH_4c and CH_4p measured in RCs (Difford et al., 2019). The initial study that investigated the phenotypic relationship between GF and sniffer measurements reported a moderate phenotypic correlation of 0.30 (transformed from r^2) on a limited number of cows (n = 20; Huhtanen et al., 2015). The phenotypic correlation estimated by Huhtanen et al. (2015), is similar to the phenotypic correlation reported in this study and the Pearson correlation estimates from the same data (Pearson's r = 0.20 (95% CI [0.15, 0.24]) and 0.19 ± 0.05 (95% CI [0.08, 0.29]) for daily and weekly means, respectively). The phenotypic correlations estimated from REML, were higher compared with the estimated Pearson correlations, which suggests the measurements are influenced by environmental factors. Environmental factors that play a role may be amplified by the fact that cows were measured at different times of day with GF and sniffers, and were not measured simultaneously. Some environmental factors can be successfully corrected for by using fixed effects in mixed models as was done in this study, where fixed effects for hour of measurement and week of measurement were included, which resulted in higher phenotypic correlations. Other techniques to improve the accuracy of sniffer systems should be further investigated, for example, by using video to record cows' head position.

3.4.3 Parameters for CO_2

The genetic correlation between CH_4p and CO_2p was 0.68 ± 0.04 for daily means and 0.65 ± 0.05 for weekly means, and the phenotypic correlations were higher and were 0.72 ± 0.01 for daily means and 0.75 ± 0.01 for weekly means (Table 3.3). The genetic correlations between CH_4c and CO_2c were high (0.93 ± 0.01, for both daily and weekly means), and so were the phenotypic correlations (0.78 ± < 0.01 and 0.84 ± < 0.01, for daily and weekly means respectively). High phenotypic and genetic correlations between CH_4 and CO_2 emissions of

dairy cows have been reported in the literature previously. A study by Difford et al. (2020) reported correlations between log-transformed CH_4c and CO_2c, and reported phenotypic correlations of 0.87 ± <0.01 and 0.96 ± <0.01, and genetic correlations of 0.96 ± 0.03 and 0.97 ± 0.03. Additionally, a study using RC measurements also reported high phenotypic correlations between CH_4p and CO_2p (0.93; Aubry and Yan (2015)). This indicates that there is a strong relationship between CH_4 and CO_2 emissions from dairy cows.

Genetic and phenotypic correlations between CH_4p measured by GF units and CO_2c measured by sniffers were moderate to low. The genetic correlations were 0.39 ± 0.16 for daily means and 0.41 ± 0.18 for weekly means, and were thus associated with large SE. The phenotypic correlations were 0.20 ± 0.04 for daily means and 0.19 ± 0.06 for weekly means. Therefore, although the genetic correlations between CH_4p and CH_4c, and between CH_4c and CO_2c were high, the genetic correlations between CH_4p and CO_2c were relatively low. This indicates that CH_4c measurements from sniffers would be a more suitable indicator for GF CH_4p, than using CO_2c measurements as a predictor. Regardless of the larger stability and less drift that we observed for measurements from the sniffer CO_2 sensor.

3.4.4 The Relationship Between CH_4 and DIM

Both the mean CH_4p measured by GF units and the CH_4c measured by sniffers increased steeply in the first weeks of lactation (Figure 3.2). Most likely this effect is caused by a low and increasing DMI that occurs in the first days of lactation (Krattenmacher et al., 2019). After the initial increase, the CH_4p measured by GF units remained stable over the further lactation, whereas the CH_4c measured by sniffers started to decrease after approximately 100 DIM. In the parameter estimations a fixed effect for DIM was fitted to correct for differences between DIM, similar to what has been used in and was recommended by previous studies (van Engelen et al., 2018). Phenotypic lactation patterns of CH_4 emissions that have been reported in the literature are inconsistent in the later weeks of lactation. The study by Bell et al. (2014b), showed that CH_4 emissions remained stable in the later weeks of lactation whereas other studies reported a decrease of CH_4 emissions in later weeks of lactation (Garnsworthy et al., 2012b; Lassen and Lovendahl, 2016). The study by Pszczola et al. (2017) split the data between first and later parity cows. The data in the study by Pszczola et al. (2017) suggested that the pattern may differ per parity, and that the decrease is only observed for first parity cows, however, this could not be confirmed by the data recorded by GF units or sniffers from this study (results not shown). The deviation in lactation patterns could have resulted from other undefined differences between the for this study recorded farms, as the majority of measurements were taken on different farms for GF units and sniffers.

3.4.5 Implications for Implementing CH_4 Emissions in Breeding Goals

Both GF units and sniffers can be used to record multiple short-term CH_4 and CO_2 measurements from the breath of dairy cows. The main difference in functionality is the

ability of GF units to record airflow, which is used in mass flux calculations to estimate CH_4p from concentration measurements (Madsen et al., 2010). Additionally, GF units record head position to ensure that the cow's muzzle is in close proximity to the air inlet. For measurements taken by sniffers, the position of the head of the cow in relation to the air inlet is unknown. A study by Huhtanen et al. (2015) showed that head movements have a high repeatability (0.74 for daily observations), indicating that there is systematic muzzle movement behaviour of cows. This systematic head movement could in theory lead to lower average concentrations that are measured for cows that frequently move their muzzle away from the air inlet. Nonetheless, the study by Huhtanen et al. (2015) found a weak correlation between muzzle position and CH_4c measured by a sniffer method including 95 cows ($r = 0.26$), and no significant relationship including only the 59 cows which had acceptable muzzle data. However, as muzzle movements have shown to be highly repeatable, the relationship between muzzle movement and CH_4c measured by sniffers should be investigated further to prevent that breeding for reduced CH_4c will result in changes in cow behaviour.

Additionally, GF units and sniffers are spot-sample techniques, and are unable to measure the total emissions of cows. At this moment, there is no technique that can measure the "true" CH_4 emissions of dairy cows. Often RCs are considered to be the gold standard for recording CH_4 emissions of individual cows, as they are able to accurately record total emissions (Hammond et al., 2016a). However, RC measurements may not reflect true CH_4 emissions (Hill et al., 2016). Cow behaviour, such as feed intake, can change when cows are isolated from the herd to be measured in a RC. Therefore, RC measurements may deviate from a cows' emissions in the herd on a commercial dairy farm. Furthermore, cows in RCs are usually recorded for a short period of time, lasting a few hours and up to 3 days, whereas CH_4 emissions do change over time. For example, by diurnal variation in CH_4 emissions (Figure 3.1), which can be a result of changes in feed intake during the day (Crompton et al., 2011). Additionally, studies by Pszczola et al. (2017), Breider et al. (2019), and Sypniewski et al. (2021) have shown that the heritability of CH_4 emission changes over a lactation. A technique that measures the true total amount of CH_4, can provide longitudinal data, and is cost-effective does not exist. The limitations in the different techniques that measure CH_4 have important implications for the application of a metric for CH_4 emissions in breeding goals that aim to reduce a cows' total emissions.

Instead of having available measurements of cows' true total CH_4 emissions, multiple measurements by different, genetically correlated, techniques and other predictors can be combined in genetic evaluations to realise the highest genetic gain and thus the highest reduction in CH_4 emissions (de Haas et al., 2017). Possible predictors can be for example rumination time (Lopez-Paredes et al., 2020), composition of the rumen microbiome (Difford, 2018), feed intake, and digestibility (de Haas et al., 2017). In this study we focused

on using sniffer CH_4c measurements as a predictor for CH_4p as recorded by GF units. The results from this study suggest that CH_4 measurements on CH_4p by GF and on CH_4c sniffers are highly genetically correlated, and indicate that selection on low CH_4c will reduce CH_4p. Because of the high genetic correlation, measurements from the two techniques could therefore be used to strengthen each other in genetic evaluations. In practice, sniffers are able to record CH_4c cost-effectively on thousands of dairy cows, which could complement the more expensive recording by GF units that measure CH_4p in grams per day. Furthermore, the high genetic correlation indicates that data could be shared between countries which have measurements available from either only GF units or sniffers. Sharing CH_4 data across countries is of interest to build a large genomic reference population. A large reference population across countries can increase the power of QTL detection and increase the accuracy of genomic prediction, as was shown in a previous project for scarcely recorded feed intake data (global Dry Matter Initiative; Banos et al., 2012; de Haas et al., 2012). An initial study by Manzanilla-Pech et al. (2021) has successfully explored combining CH_4 measurements across countries and from different methods of measuring CH_4 (i.e., GF, sniffer, and SF_6), although more data are needed to better disentangle the use of different methods in different countries which was in some cases confounded.

3.5 Conclusions

To phenotype cows for CH_4 emissions, many different methods have been developed and are currently used in research practices. To be able to judge the similarity and repeatability between CH_4 measurements of different recording techniques, the genetic correlation can be a useful metric. Combining measurements by highly genetically correlated CH_4 recording techniques can help to enlarge existing datasets, for example by sharing data across countries, which is needed for accurate genetic evaluations. In this study, we have shown that the genetic correlation between CH_4p measured by GF units and CH_4c measured by sniffers was high (0.71 ± 0.13 for daily means and 0.76 ± 0.15 for weekly means). In addition, the heritability for CH_4p recorded by GF units was moderate and was similar to the heritability estimated for CH_4c measured by sniffers, 0.19 ± 0.02 and 0.33 ± 0.04 for daily and weekly means for GF, and 0.18 ± 0.01 and 0.32 ± 0.02 for daily and weekly means for sniffers, respectively. These results indicate that genetic selection on low CH_4c (ppm) recorded by the cheaper sniffer method, will result in reduced CH_4p (grams/day).

Acknowledgements

This study was financially supported by the Climate Approach project funded by the Dutch ministry for agriculture, nature and food quality (LNV; project number BO-53-003) and was supported by the TKI Agri & Food project LWV19155 and the partners CRV and FrieslandCampina. The authors gratefully acknowledge the farmers for participating in this

research, Johan Ploegaert, Theo van Hattum, and Henk Gunnink (Wageningen Livestock Research, Wageningen, the Netherlands) for installing and maintaining the sniffers and GreenFeed units, Hendrik Jan van Dooren (Wageningen Livestock Research, Wageningen, the Netherlands) for setting up data collection for sniffers, and CRV (Arnhem, the Netherlands) for providing the pedigree and genotype data. The authors have not stated any conflicts of interest.

A1. Residual correlations between methane (CH_4) and carbon dioxide (CO_2) recorded by GreenFeed (GF, production: CH_4p in g/day) units or sniffers (concentration: CH_4c in ppm) and averaged per day or per week (± standard error).

	GF CH_4p day	GF CO_2p day	GF CH_4p week	GF CO_2p week	Sniffer CH_4c day	Sniffer CO_2c day	Sniffer CH_4c week	Sniffer CO_2c week
GF CH_4p day		0.70 ± <0.01	0.43 ± 0.01[2]	0.29 ± 0.01	0.39 ± 0.02	0.19 ± 0.03	0.40 ± 0.02	0.21 ± 0.03
GF CO_2p day			0.33 ± 0.01	0.47 ± <0.01[2]	0.35 ± 0.03	0.24 ± 0.03	0.42 ± 0.02	0.31 ± 0.03
GF CH_4p week				0.70 ± <0.01	0.23 ± 0.03	0.07 ± 0.03	0.42 ± 0.02	0.21 ± 0.03
GF CO_2p week					0.14 ± 0.03	0.06 ± 0.03	0.29 ± 0.03	0.18 ± 0.03
Sniffer CH_4c day						0.72 ± <0.01	0.52 ± <0.01[2]	0.39 ± <0.01
Sniffer CO_2c day							0.42 ± <0.01	0.54 ± <0.01[2]
Sniffer CH_4c week								0.77 ± <0.01
Sniffer CO_2c week								

[2] Estimate with the highest likelihood, but with convergence problems due to closeness to unity of the correlation

4

Genetic parameter estimates for methane emission during lactation from breath and potential inaccuracies in reliabilities assuming a repeatability versus random regression model

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Published in Journal of Dairy Science. Volume 107, Issue 8, Pages 5853-5868, August 2024

DOI https://doi.org/10.3168/jds.2024-24285

Abstract

Methane emissions will be added to many national ruminant breeding programmes in the coming years. Little is known about the covariance structure of CH_4 traits over a lactation, which is important for optimizing recording strategies and establishing optimal genetic evaluation models. Our aim was to study CH_4 over a lactation using random regression (RR) models, and to compare the accuracy to a fixed regression repeatability model under different phenotyping strategies. Data were available from repeated measurements of CH_4 concentrations (ppm) recorded in the feed bins of milking robots on 52 commercial dairy farms in the Netherlands. In total, 36,370 averaged weekly records were available from 4,664 cows. Genetic parameters were estimated using a fixed regression model, and a RR model with first- to fifth-order Legendre polynomials for the additive genetic and within-lactation permanent environmental effect. The mean heritability was 0.17 ± 0.04 and the mean within-lactation repeatability was 0.56 ± 0.03. The genetic correlations between DIM were high and ranged from 0.34 ± 0.36 to $1.00 \pm <0.01$. Permanent environmental correlations showed large deviations and ranged from -0.73 ± 0.08 to $1.00 \pm <0.01$. With a large number of full lactation daughter CH_4 records per bull, the reliability was not sensitive to using the fixed versus the RR model. However, when shorter periods were recorded at the start and end of the lactation, the fixed regression model resulted in a loss of reliability up to 28% for bulls. Assuming the fixed model when the true (co)variance structure is reflected by the RR model, more than twice as long of a recording from the start of lactation was required to achieve maximum reliability for a bull. Thus, a too simplistic model could result in implementing too little recording, and in lower genetic gains than predicted from the reliability.

Key words: methane, breeding, random regression, dairy cows

4.1 Introduction

Enteric fermentation by ruminants is the source of approximately 6% of all anthropogenic greenhouse gas emissions, and ruminant emissions are expected to increase because of the increasing global demand for meat and milk leading to more ruminants being kept worldwide (Beauchemin et al., 2020). Ruminant greenhouse gas emissions, with CH_4 as the main contributor, have a significant effect on climate change, as CH_4 has a global warming potential approximately 27 times greater than CO_2 over a 100-yr lifespan (IPCC, 2021). The application of selective breeding to lower the environmental impact of enteric CH_4 emissions from cattle is a topic of worldwide interest because it offers a cost-effective solution, and the effect is permanent and cumulative (Lassen and Difford, 2020).

To apply selective breeding, models that accurately estimate breeding values for individual animals and correlations with other important breeding goal traits will be needed. The goal of selective breeding is to reduce CH_4 emissions along full lactations. However, recording CH_4 is challenging and requires high levels of labour, knowledge, and expensive specialised equipment, and measurements of the total amount of CH_4 that is emitted during full lactations are not easily available for large number of cows (Garnsworthy et al., 2019). Equipment is often routinely exchanged between farms for short periods of time to maximise the number of cows that can be recorded. As a result, it is important to determine the optimal moment and length of recording to ensure highest accuracy with CH_4 emissions for the full lactation. In addition, the best model to be used for genetic evaluations is critically dependent on the genetic covariance structure of methane records collected during part of the lactation. Most initial studies that estimated genetic parameters for CH_4 emissions of dairy cattle applied repeatability models (Lassen and Lovendahl, 2016; van Engelen et al., 2018), which generally assume that the genetic correlation between records taken during different stages of a lactation are equal to unity. However, previous studies that used random regression (RR) models, which allow for different genetic variances and covariances between time points, have shown that the repeatability and heritability of CH_4 concentrations measured from breath change over a lactation (Breider et al., 2019; Manzanilla-Pech et al., 2022a; Pszczola et al., 2017; Sypniewski et al., 2021). Differences in variances and correlations over the lactation may have important implications for the genetic gains that can be predicted for, and expected from, future breeding pro- grams that aim to reduce CH_4 emissions. For example, the genetic correlation between lactation stages is a good indicator for whether CH_4 concentrations should be modelled with RR, as multiple traits, or if using a single trait in a repeatability model is sufficient.

For the repeatability of daily CH_4 concentrations, Pszczola et al. (2017) reported a steep decrease in repeatability in mid lactation, ranging from 0.40 to 0.18, whereas Manzanilla-Pech et al. (2022a) reported the highest repeatabilities in mid lactation for weekly CH_4

concentrations, ranging from 0.63 to 0.86 (median SE = 0.03). For the heritability of CH_4 concentrations, Manzanilla-Pech et al. (2022a) and Pszczola et al. (2017) reported relatively stable heritabilities over a lactation, which reached the maximum in mid lactation of 0.10 to 0.28 (median SE = 0.05) and 0.27 ± 0.12 to 0.30 ± 0.08, respectively. In contradiction, Breider et al. (2019) reported that heritabilities for weekly CH_4 concentrations were lowest in mid lactation and reached the maximum in late lactation (h^2 = 0.12 ± 0.16 to 0.45 ± 0.11), and Sypniewski et al. (2021) reported a steady increase of heritability of daily CH_4 concentrations over the lactation (h^2 = 0 to 0.14), with the heritability being zero at the start of the lactation. Thus, a consensus has not been reached to date on the shape of the curve of the repeatability or the heritability estimated over a lactation. Differences in results may have arisen from including records on a relatively small number of cows, ranging from 184 to 575, coming from at most 2 farms. As a result, when reported, standard errors were high. Furthermore, for 3 of the 4 studies, the data recording period did not cover a full lactation. As explained above, being able to model the covariance structure over the full lactation correctly helps to draw inferences from longitudinal data. Therefore, our objective was to study the heritability and repeatability of measured CH_4 concentrations over a lactation using a RR model, and to compare the accuracy of using a fixed regression repeat- ability model or the RR model under different phenotyping strategies. For the analyses we used a novel dataset from emissions measured on the largest number of cows to date from across the Netherlands, which has previously only been analysed using repeatability models (van Breukelen et al., 2022).

4.2 Materials and Methods

4.2.1 Methane recording

Data were collected on 7,097 Holstein cows, from 54 farms (Table 4.1), using non-invasive sniffers (WD-WUR v1.0 and v2.0, manufactured by Carltech BV), that sampled CH_4 and carbon dioxide (CO_2) concentrations (ppm) from the feed bin of automated milking systems (AMS). Ethical approval was not needed for this study because no animal procedures were performed. The number of cows recorded per farm ranged from 43 to 299. Various types of AMS systems were present in the study, manufactured by: DeLaval (DeLaval BV), Fullwood (Fullwood Packo BV), GEA (GEA Group), Lely (Lely Industries NV), and SAC (SAC BV). On each farm, at most one AMS unit was equipped with a sniffer. This strategy was decided on, to maximise the numbers of farms with phenotyping, and thereby maximizing the number of cows that were phenotyped. The sniffers were calibrated to measure CH_4 concentrations (CH_4c) in a range of 0 to 2,000 ppm. Measured concentrations can be used as a proxy for CH_4 emissions (g/day), similar to what has been reported in previous studies (Difford et al., 2020; van Breukelen et al., 2023). Data were recorded between March 2019 and March 2023. Between March 2019 and February 2021, v1.0 sniffers were used of which the data collection

process is described in more detail in van Breukelen et al. (2022). Between December 2021 and March 2023, v2.0 sniffers were used. The two versions of sniffers functioned similarly, however, the v2.0 sniffers measured concentrations every five seconds, opposed to the longer recording intervals of the v1.0 sniffers (ranging from 10 to 35 seconds). Furthermore, the v2.0 sniffers had improvements to the housing and data sharing, which benefitted the ongoing data collection in the barn environment but did not change how CH_4 was measured.

Table 4.1. The number of farms, number of recorded cows, and total number of records for visit and weekly mean methane CH_4 concentrations, after each step of data editing

	N farms	N cows	N records
Visit CH_4	54	7,097	661,917
Weekly CH_4	53	4,935	38,858
Excluding > 405 days in milk	53	4,869	38,075
Excluding < 75% Holstein	52	4,664	36,370

4.2.2 Data editing

The sniffer records were filtered to exclude data which were biologically improbable, for example due to blocking of the sampling tube in the AMS feed bin by dust from pellets. To do so, for each hour within a farm, data recorded within that hour would be discarded if: 1) the mean was below 30 ppm CH_4, 2) the inter quartile range was below 200 ppm CH_4, 3) the maximum was above 3,500 ppm CH_4, or 4) if at least 30% of the data would fall within the range of the first and second mode, plus and minus 10 ppm CH_4. Furthermore, individual outliers were discarded, which were defined as being lower than -200 ppm CH_4, or values outside of the upper and lower 0.001^{th} quantile. A threshold below zero was used, because sensors could measure below zero if the calibration drifted. Nonetheless, calibration lines would remain linear and could therefore still provide important information about variation in emissions on that farm. After filtering, background concentrations were estimated from the 0.001 lowest quantile of each day and subtracted from each measurement.

The timestamps (on the level date-time, with time as hours, minutes, and seconds) from sniffer data and AMS data (also provided by CRV) were used to connect cow ID to each individual sniffer record. The AMS and v2.0 sniffers automatically synchronised to real time through the Odido (Odido Netherlands BV) mobile network, to prevent drift of the clocks; for the data recorded by v1.0 sniffers the alignment process is described in van Breukelen et al. (2022). In addition, the alignments were confirmed visually for each farm, by observing if CH_4 concentration would increase after the start of a milking and remain low when no cow was in the AMS. After the alignment, the mean CH_4 concentration per AMS visit was calculated for each individual visit. In calculating the mean, only measurements recorded

from the first and up to the fifth minute of milking were used. The other measurements were discarded to account for the delay in the air sample reaching the sniffer, and to exclude records when cows are likely to have finished eating the pellets provided in the AMS feed bin, which makes it more likely that cows move the head away from the sampling inlet. Thereafter, means that were derived from less than two and a half minutes of milking were discarded, to ensure that multiple records were collected during the milking visit, and that these records included belching events (van Soest, 1994). The aligned data were averaged per AMS visit and contained 661,917 records of mean CH_4c (Table 4.1).

A linear model was used to correct each record for diurnal variation in measured CH_4 concentrations within farm, with a Fourier series approach (Lassen and Lovendahl, 2016; Lovendahl and Bjerring, 2006), using the following model:

$$y_{ij} = \mu + Farm_i . \sum_{j=1}^{1} (\sin j\theta 2\pi + \cos j\theta 2\pi) + e_i \qquad (1)$$

where y_{ij} is the phenotype for the mean CH_4c per AMS visit; Farm is the fixed effect for the i^{th} farm; θ is a decimal fraction of the time of measurement, following a 24-hour diurnal cycle (i.e., θ = hour at measurement / 24), where j is the order of regression; and e is the residual error, following $e_i \sim N(0, \mathbf{I}\sigma^2_e)$, where $\mathbf{I}$ represents the identity matrix and σ^2_e the residual variance. To derive the corrected estimates for each visit, the estimated fixed effects were subtracted from the corresponding measurement.

The mean CH_4c per AMS visit were then summarised as weekly mean concentrations (Table 4.1). If the weekly mean of a cow consisted of less than seven AMS visits, then the weekly record was discarded. Two farms had a sniffer installed for only a short period of time and had issues with data collection, and because of the limited number of records per cow weekly means were not available, and the two farms were discarded (n=52). On average, a weekly mean record consisted of 11 recorded visits (min-max: 7-33). For each weekly measurement, the associated days in milk were reported as the first day that was included in the weekly measurement. Large differences were observed in the mean and standard deviation of records taken with the different versions of sniffers. Therefore, CH_4c was scaled to a standard deviation of one and centred within version of sniffer, by subtracting the mean of all records, measured with the corresponding sniffer version, from each measurement and dividing the result by the standard deviation of all measurements with the corresponding sniffer version (e.g. a CH_4c record taken by a v1.0 sniffer was scaled with the overall v1.0 sniffer mean and standard deviation). The data were not log transformed, as this had little impact on normality of the data in previous analyses (van Breukelen et al., 2022), and the residuals from the

analyses appeared normally distributed. For the genetic analyses, the data set was filtered to include only records up to 405 days in milk (DIM) and the animals that were at least 75% Holstein (Table 4.1). The number of recorded and cows after each filtering step, and the final numbers used for the analyses are reported in Table 4.1. The number of records and average CH_4c (before standardisation) per DIM are visualised in Figure 4.1. The reduced number of records in late lactation appear to be related to fewer visits to the milking robot, which aligns with the natural decline in milk yield for cows later in their lactation, possibly in combination with other factors. On average, the final data set included 8 weekly mean CH_4c records per cow, with minimum of 1 record and a maximum of 37 records. Per farm, on average 19 weeks were recorded, with a minimum of 1 week and a maximum of 63 weeks.

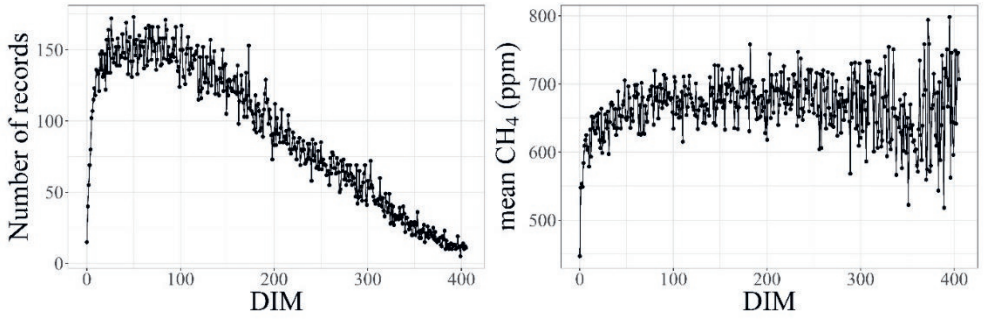

Figure 4.1. The total number of records (left) and phenotypic pattern of weekly mean methane (CH_4) concentration (right) over a lactation as days in milk (DIM)

4.2.3 Pedigree data

Pedigree and other cow information were provided by CRV (Arnhem, the Netherlands). The pedigree was pruned to include all cows with a CH_4 record and their ancestors using the R-package "pedigreeTools" in R v4.2.0. The pruned pedigree was 25 generations deep and contained 48,926 animals.

4.2.4 Genetic parameter estimation

To investigate if measurements from the two sniffer versions are interchangeable, fixed regression bivariate analyses were performed that applied CH_4c from the v1.0 or v2.0 sniffers as different traits, to estimate genetic correlations, using the model defined in Equation *(2)* as a bivariate model. The bivariate model excluded the across parity permanent environmental effect due to convergence issues. Nonetheless, a univariate model with the across parity permanent environmental random effect was also used to estimate the heritability for each sniffer version. Since this effect was included in the further analysis.

Thereafter, a single-trait RR model was used to estimate variance components, using a restricted maximum likelihood method in ASReml 4.2ng (Gilmour et al., 2015). Different orders of Legendre polynomials for the random genetic and permanent environmental effect were fitted, ranging from the 0th to the 5th order, and compared using the Loglikelihood, Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC). The model using Legendre polynomials of the 0th order is equal to using a fixed regression repeatability model. The following model was used to estimate genetic parameters:

$$y_{ijlk} = \mu + HYW_i + Par_j.\sum_{k=0}^{3} \emptyset(t)_{lk}\beta_k + Breed.\sum_{k=0}^{2} \emptyset(u)\beta + \sum_{k=0}^{n} \emptyset(t)_{lk}a_{lk} + \sum_{k=0}^{n} \emptyset(t)_{lk}pepar_{lk} + pe_l + e_{ijl} \quad (2)$$

where y_{ijlk} is the scaled and centred phenotype for the mean CH_4c per week; μ is the mean; *HYW* is the fixed effect for the interaction of herd, year, and week of measurement *i*; *Par* is the fixed effect for parity (*j* = 1 to 4, where 4 includes parity four or higher), and is fitted as an interaction with β, which is a fixed regression coefficient with third-order Legendre polynomials, measured at *t* DIM, for the k^{th} regression coefficient of animal *l*; *Breed* is the fixed effect for the second breed, and is fitted as an interaction with β, which is a fixed regression coefficient with second-order Legendre polynomials measured as a breed fraction *u* that is other than Holstein derived from the pedigree; a_{lk} and $pepar_{lk}$ are RR coefficients for the genetic effect and the permanent environmental effect within parity, respectively, and $\emptyset$ is the term for the nth order Legendre polynomial (ranging from zero to five) at *t* DIM; *pe* is the random permanent environmental effect across lactations; and e_{ijl} is the random residual. The residual error was assumed to have heterogeneous variances and was divided into five classes for DIM (0-59, 60-119, 120-239, 240-359, and 360-405).

Although Legendre polynomials give a rank reduction compared with a full rank matrix between all DIM, higher order Legendre polynomials might still give convergence issues in ASReml. This is due to the average information algorithm struggling with close to non-positive definite matrices, and the expectation-maximization algorithm being notoriously slow. Therefore, factor analytical modelling, using XFA (extended factor analysis) inflation factors, was used in ASReml, to decompose and reduce the rank of the variance-covariance matrix of the Legendre polynomials (Thompson et al., 2003). The results reported in the

paper applied a number of XFA equal to the order of Legendre polynomials, except when fitting polynomials of the fourth and fifth order. Because of convergence issues, here the third and fourth XFA were fitted for the models with fourth, and fifth order Legendre polynomials, respectively.

Estimated (co)variance components were used together with the Legendre polynomial coefficients at each DIM to estimate the full (co)variances matrices and the genetic parameters (heritability, repeatability, and correlations between DIM) and their approximate standard errors (SE), as described by Fischer et al. (2004). In short, the genetic, permanent environmental, and phenotypic (co)variances were estimated by:

$$\mathbf{G} = \mathbf{\Phi K \Phi}' \text{ and} \quad (3)$$

$$\mathbf{PE} = \mathbf{\Phi KPE \Phi}' \text{and} \quad (4)$$

$$\mathbf{P} = \mathbf{G} + \mathbf{PE} + \sigma_e^2 \quad (5)$$

where **G** is the genetic (co)variance matrix; **PE** is the permanent environmental (co)variance matrix, to which the across parity permanent environmental variance was added to all (co)variance elements in the matrix; and **P** is the phenotypic (co)variance matrix per DIM (*n*n*, where *n* is the level of DIM, consisting of 58 classes of seven DIM, up to 400 DIM); **Φ** is a matrix of order *t*n*, where *t* is equal to the number of orthogonal polynomial coefficients; **K** and **KPE** are matrices of order *t*t*, which contain the estimated covariance functions that describe the genetic (co)variance components and permanent environmental (co)variance components, respectively, for each RR coefficient; and σ^2_e is the residual variance that is added to the diagonal of the phenotypic (co)variance matrix, with residual (co)variances assumed to be zero.

The heritability (h^2) was defined as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{wpe}^2 + \sigma_{ape}^2 + \sigma_e^2} \quad (6)$$

The within lactation repeatability (*r*) was defined as:

$$r = \frac{\sigma_a^2 + \sigma_{wpe}^2}{\sigma_a^2 + \sigma_{wpe}^2 + \sigma_{ape}^2 + \sigma_e^2} \quad (7)$$

And the across lactation repeatability (*r*) was defined as:

$$r = \frac{\sigma_a^2 + \sigma_{ape}^2}{\sigma_a^2 + \sigma_{wpe}^2 + \sigma_{ape}^2 + \sigma_e^2} \quad (8)$$

where σ_a^2 is the additive genetic variance; σ_{wpe}^2 is the permanent environmental variance within lactations; σ_{ape}^2 is the permanent environmental variance across lactations; and σ_e^2 is the residual variance.

4.2.5 Selection index calculations

To predict the reliability using genetic evaluation models assuming different orders of Legendre polynomials for the random effects, the associated selection index coefficients were estimated, following:

$$\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}\mathbf{v} \quad (9)$$

where **b** is the selection index coefficient, $\mathbf{P}^{-1}$ is the inverse of the phenotypic (co)variance matrix among observations in the selection index (n*n, where *n* consists of 58 potential classes of weeks in milk where recording takes place), **G** is the *n***m* matrix of genetic (co)variance matrix among the *n* weeks in P and all *m* traits in the aggregate genotype (where *m* consists of 58 classes of weeks in milk), and **v** is a vector with weights, which were kept equal to for al 58 weeks in milk. Because for all orders the smallest eigenvalues of the **G** matrix were slightly negative, the matrix was first made positive semidefinite. This was done by changing negative eigenvalues to 0.001. Thereafter, the matrix was again constructed by multiplying the eigenvectors with the transformed diagonal of the matrix and the transpose of the eigenvectors. Nonetheless, the non-positive definite matrices yielded similar results (not shown).

Thereafter, the accuracies of the indexes (r_{HI}) were estimated using the following formula:

$$r_{HI} = \frac{\mathbf{b}'\mathbf{G}\mathbf{v}}{\sqrt{\mathbf{b}'\mathbf{P}\mathbf{b}\ \mathbf{v}'\mathbf{C}\mathbf{v}}} \quad (10)$$

Which applied the parameters from the previous formula, and the matrix **C** which is a m**m* matrix with genetic (co)variances in the aggregate genotype. From this, reliabilities were predicted for the different models as the squared accuracies. These reliabilities reflect the model reliabilities that would be published for breeding values given that the assumed model was used (regardless of the true genetic parameters).

The reliability was also calculated for an additional scenario, i.e. where the repeatability model was used, but in fact the estimated parameters from the RR reflect the true parameters more closely. In that scenario the b-values reflect a "sub-optimal" index using the fixed regression repeatability model, whereas the RR model gives the "optimal" index.

Optimal indices and models also depend on the recording strategy, and the number of records, since **P** will be affected. Therefore, several scenarios were evaluated. For the first set of scenarios, it is assumed that for each recorded week in the lactation one record is available per cow (scenarios "Cows"). The **P** matrices simply reflect the phenotypic (co)variances in this case. In the second set of scenarios, the **P** matrices were replaced with the (co)variances of the **G** matrix, simulating sires with a large number of daughters recorded, (scenario "Bulls"). The large number of records provide close to the true genotype for a bull at the recorded moments.

Taking the two models, and the Cow and Bull situation, scenarios were investigated where observations were only available for parts of the lactation. This is relevant for example when cows are phenotyped for CH_4 emissions with expensive equipment, which is routinely exchanged between farms for short periods of time. The scenarios were: 1) with records on different parts of the lactation, where always eight weeks (56 days) were recorded sequentially; 2) with records on different parts of the lactation, where always three weeks (21 days) were recorded sequentially; and (3) starting with one observation at the start of the lactation, which cumulatively increased by one week of recording until 400 DIM was reached, again changing the **P** and **G** matrices based on the DIM with records.

4.3 Results

4.3.1 Exploratory analyses

Before standardisation, the mean CH_4c of v1.0 sniffer sensors was 512 ppm, with a standard deviation of 172 ppm, and from v2.0 sniffers the mean was 719 ppm, with a standard deviation of 313 ppm (Table 4.2). The coefficients of variation were 34% and 44%, for mean CH_4c measured by the v1.0 and v2.0 sniffer sensors, respectively. The overall mean CH_4c per week was 668 ppm, with a standard deviation of 299 ppm. For the overall mean, the coefficient of variation was 45%. After standardisation and filtering of the data, the mean scaled CH_4c of v1.0 sniffer sensors was 3.74, with a standard deviation of 1.01, and from v2.0 sniffers the mean scaled CH_4c was 2.03, with a standard deviation of 1.00. The overall mean scaled CH_4c per week was 3.7, with a standard deviation of 1.24. The total number of recorded DIM for an individual cow ranged from 1 to 271, and the total number records per DIM are plotted in Figure 4.1.

Table 4.2. The mean, standard deviation (SD), minimum, maximum, and coefficient of variation (CV) of the weekly mean methane concentration (CH_4c) phenotype in parts per million (ppm), and after standardisation, recorded by two different versions of sniffers (v1.0 and v2.0) and as all measurements combined

		Mean	SD	Min	Max	CV
CH_4c (ppm)	v1.0	512	172	86	1350	34%
	v2.0	719	313	62	2057	44%
	Combined	668	299	62	2057	45%
Standardised CH_4c	v1.0	3.74	1.01	1.25	8.63	27%
	v2.0	2.06	1.00	-0.04	6.34	49%
	Combined	2.47	1.24	-0.04	8.63	50%

From the data including only measurements from v1.0 sniffers, the heritability and repeatability were 0.27 ± 0.03 and 0.62 ± 0.01, and for v2.0 they were 0.37 ± 0.03 and 0.76 ± 0.01. The genetic corelation between the two sensors was 0.99 ± 0.09. Nonetheless, there was a considerable difference in the estimated variance components. For v1.0 and v2.0 data, respectively, the phenotypic variances were 15,759 ± 468 and 52,662 ± 1,208, and the genetic variances were 4,180 ± 620 and 19,368 ± 1,936. Thus, in general the measurements taken with v2.0 sniffers were associated with higher variances compared with v1.0 sniffers, justifying the need to standardise the sniffer data to a common phenotypic variance. But because of the high genetic correlation and not majorly different heritabilities, it was decided to further analyse the data using univariate models, where we assume that after phenotypic standardisation the records taken by either version of sniffer is genetically the same trait.

4.3.2 Order of the random effects

The random effects of the RR model were modelled with different orders or Legendre polynomials (0 to 5^{th}). The fit of the models was investigated based on the Log Likelihood, AIC and BIC. The models that applied higher orders of Legendre polynomials had consecutively better goodness of fit, indicated by the lower AIC and BIC, and the higher Log Likelihood (Table 4.3).

Table 4.3. The Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and log likelihood (LogL) for the random effects fitted with Legendre polynomials of the 0th (Leg 0), 1st (Leg 1), 2nd (Leg 2), 3rd (Leg 3), 4th (Leg 4), and 5th (Leg 5) order

	AIC	BIC	LogL
Leg 0	-16986.4	-16918.5	8501.2
Leg 1	-19714.7	-19604.4	9870.4
Leg 2	-21060.9	-20933.6	10545.4
Leg 3	-21810.3	-21632.1	10926.1
Leg 4[1]	-22402.9	-22123.0	11234.5
Leg 5[2]	-22762.6	-22431.8	11420.3

[1,2] All models are fitted with the same number of XFA factors as the number of orders, except [1] and [2] which were fitted with XFA 3 and XFA 4, respectively

4.3.3 Genetic parameter estimates

The estimated heritability, using a fixed regression repeatability model was 0.34 ± 0.02, and the repeatability was 0.73 ± 0.01. However, when including an across lactation permanent environmental effect (as is applied in the further analyses), the heritability and repeatability were lower. Here, the heritability was 0.18 ± 0.03, and ranged between 0.17 and 0.18 for the different groups of residual variances. The average within lactation repeatability was 0.48 ± 0.03 and ranged between 0.46 and 0.49 for different groups of residual variances. The average across lactation repeatability was 0.41 ± 0.02 and ranged between 0.39 and 0.42 for different groups of residual variances.

The lactation pattern for the heritability of the random effects modelled with different orders of Legendre polynomials was similar between orders (Figure 4.2, top). The heritability of the fixed regression repeatability model (Leg 0) was higher in the beginning and end of the lactation, whereas the models with first or higher order Legendre polynomials had a higher heritability throughout mid lactation. For the fourth order RR model, the heritability of weekly mean CH_4c was on average 0.17 ± 0.04 and increased steeply in the first 130 DIM. The maximum heritability was 0.21 ± 0.03 at 134 DIM. Thereafter, the heritability decreased, first slowly and then steeply, to a minimum of 0.10 ± 0.04 at 358 DIM. The heritability of the model with Legendre polynomials of the fifth order deviated from the other estimates at the end of the lactation, where they peaked at a level of 0.33 at 393 DIM.

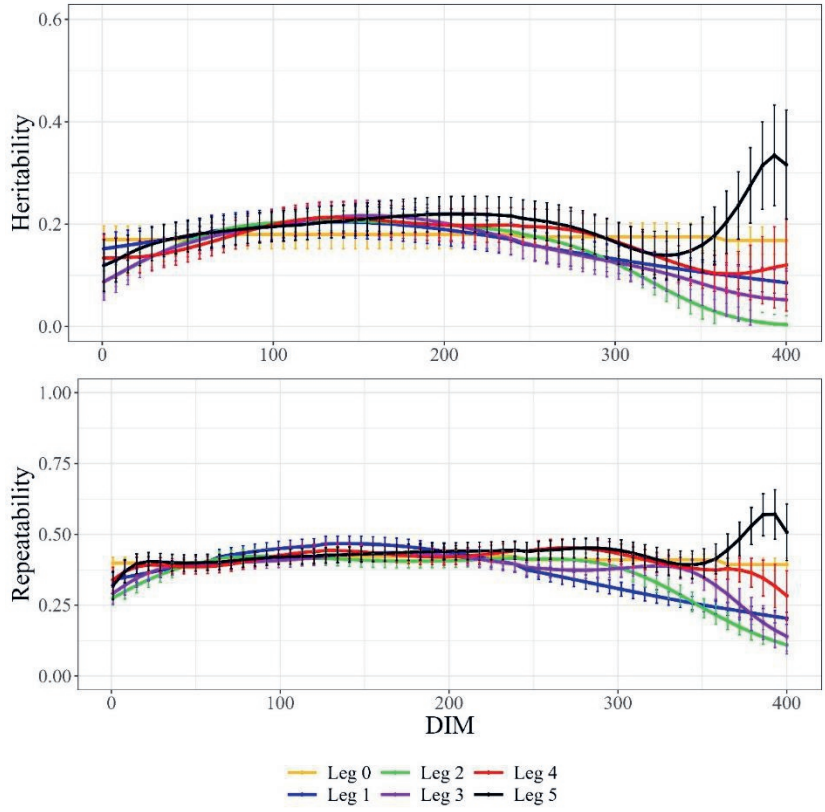

Figure 4.2. Heritability (top) and within lactation repeatability (bottom) over a lactation (DIM; days in milk) for the mean CH_4 concentration per week, with standard errors, using a random regression model with random effects fitted with Legendre polynomials of the 0th (Leg 0; yellow), 1st (Leg 1; blue), 2nd (Leg 2; green), 3rd (Leg 3; purple), 4th (Leg 4; red), and 5th (Leg 5; black) order

Similarly, the lactation pattern for the within lactation repeatability of the random effects modelled with different orders of Legendre polynomials was similar and were within a range of 0.45 to 0.83 (Figure 4.2, bottom). The repeatability from the fixed regression repeatability model (Leg 0) was lowest throughout the full lactation. Throughout mid lactation, the repeatability of the second, third, fourth and fifth order models were close to identical, whereas the first order model had a lower repeatability between 75 and 210 DIM, and a higher repeatability after 250 to 380 DIM. For the fourth order RR model, the repeatability remained stable during mid lactation, at approximately 0.56 ± 0.03. The minimum repeatability was 0.49 ± 0.04 at 351 DIM, and the end of the lactation the repeatability peaked to 0.70 ± 0.04 at 400 DIM. The across lactation repeatability of the fourth order model ranged from 0.28 ± 0.09 to 0.45 ± 0.03, with an average repeatability of 0.41 ± 0.03.

The underlying variance estimates, using the second to the fifth order models, are similar (Figure 4.3). The additive genetic variance estimates of the third to fifth order models spiked at the end of the lactation after approximately 320 to 375 DIM. This spike was the largest for the model with fifth order regressions. The within parity permanent environmental variance was similar for the first to the fifth order and spiked at the end of the lactation. For the model of the fifth order, the permanent environmental variance did not increase greatly at the end of the lactation and had a maximum of 0.22.

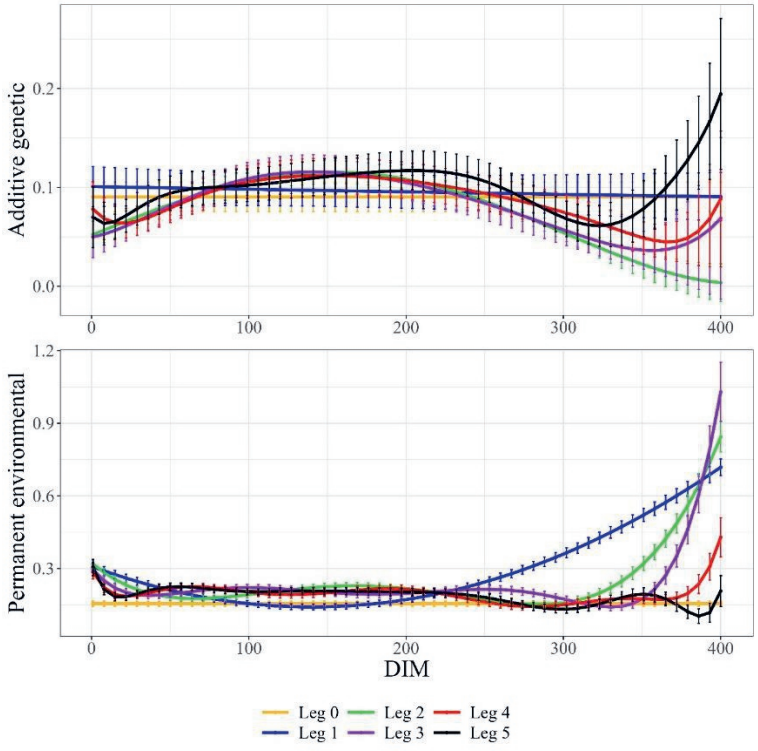

Figure 4.3. The additive genetic (top) and within lactation permanent environmental variance (bottom) over a lactation (DIM; days in milk) for the mean CH_4 concentration per week, with standard errors, using a random regression model with random effects fitted with Legendre polynomials of the 0th (Leg 0; yellow), 1st (Leg 1; blue), 2nd (Leg 2; green), 3rd (Leg 3; purple), 4th (Leg 4; red), and 5th (Leg 5; black) order

The residual variance, which was modelled in five classes of DIM, consistently decreased for higher orders of Legendre polynomials that were fitted (Figure 4.4). Especially the fixed regression repeatability model (Leg 0) and the model using a first order RR had somewhat higher residual variances. The estimates for second to fifth order RR were similar, especially during mid lactation.

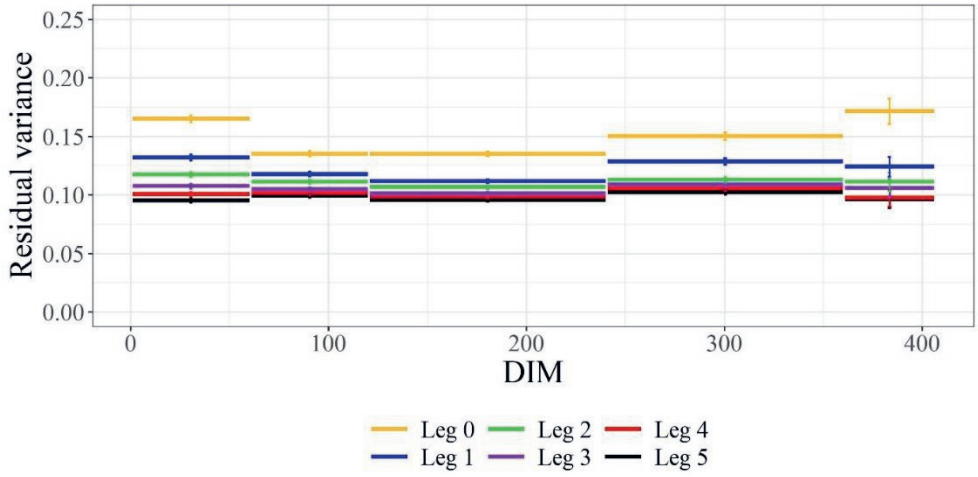

Figure 4.4. The residual variances over a lactation (DIM; days in milk) for the mean CH_4 concentration per week, with standard errors, using a random regression model with random effects fitted with Legendre polynomials of the 0th (Leg 0; yellow), 1st (Leg 1; blue), 2nd (Leg 2; green), 3rd (Leg 3; purple), 4th (Leg 4; red), and 5th (Leg 5; black) order

4.3.4 Genetic correlations between DIM

The genetic correlations for the mean CH_4c per week between DIM were high and many approximated one, except for measurements at the start and end of the lactation (Figure 4.5, left). The genetic correlation was on average 0.91 ± 0.08. The lowest correlation was 0.34 between the 302nd and 400th DIM and had a large SE of 0.36.

The within lactation permanent environmental correlations between mean CH_4c per week between DIM were high between measurements taken close in time (up to 1.00 ± <0.01). Whereas the permanent environmental correlations were low, and in some cases negative, for measurements taken further apart in time (up to -0.73 ± 0.08, between 1 and 393 DIM) (Figure 4.5, right). The mean permanent environmental correlation was 0.39 ± 0.06.

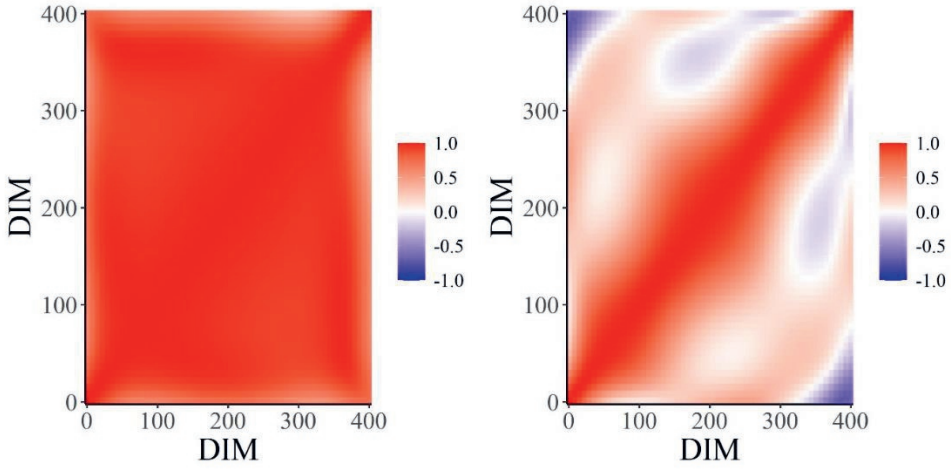

Figure 4.5. The additive genetic correlation (left) and within lactation permanent environmental correlation (right) between different stages of the lactation as days in milk (DIM) for weekly mean CH_4c, modelled with a fourth order random regression model

4.3.5 Selection index calculations

Selection index coefficients and the model reliability of the index (i.e. published breeding values) were predicted to evaluate the effect of using a fixed or a RR model in the genetic evaluations. Firstly, assuming that recording happened during the whole lactation period. The reliability of the selection index (r^2) in the scenarios for cows (using the phenotypic (co)variance matrix as **P**) was highest at 0.43 for the model using fifth order RR (Table 4.4). This was followed by the model with second, third, and fourth order regressions at 0.29. The model with the lowest reliability was the fixed regression repeatability model (Leg 0) at 0.24. Thus, the published reliability will be lower with the fixed regression model. However, the reliability, when using the b values for the fixed regression model (sub-optimal), Leg 0, when assuming the (co)variance estimates from the fifth order RR model was still 0.31. Thus, when using the fixed regression repeatability model in the genetic evaluation model, only 71% of the reliability would be realised when we assume that the fifth order RR model yields true (co)variances. However, for the first to fourth order RR model, the difference with the fixed regression model were smaller, and ranged from 92% to 98%.

The reliabilities when we assumed a large number of daughter records are available for each individual bull (the bulls scenario) were all one. Similarly, when using the b values of the fixed regression repeatability model with the genetic (co)variance matrix instead of the phenotypic (co)variance matrix (sub-optimal) the repeatabilities in all scenarios were also one, thus there was little loss using the simple repeatability model.

The results reported above assume that full lactations are recorded for individual cows. In future phenotyping strategies, it is possible that data on weekly CH_4c, or other CH_4 traits, are not available throughout a full lactation, but still the interest is in a breeding value predicting the full lactation. Therefore, we predicted the reliability of the breeding values for the full lactation, with limited data available during various parts of the lactation (using a fourth order RR model, and a fixed regression repeatability model). Overall, reliabilities for the breeding values from measurements taken during mid lactation (50 to 302 DIM) were predicted to be higher than the reliabilities of measurements taken at the start and end of the lactation (Table 4.5). When using the fixed regression repeatability model, and assuming the (co)variances estimated from the RR model are reality (sub-optimal), when measuring during the first 57 DIM only 93% of the reliability would be realised.

Table 4.5. The reliability of the selection index (r^2) with phenotypes only being available within sequential ranges of 56 days in milk (DIM) (eight weeks), when using the phenotypic (co)variance matrix (Cows), and genetic (co)variance matrix as phenotypes (Bulls) for the random regression model of the fourth order (optimal (opt)), with using the selection index coefficients coming from the fixed regression repeatability model (sub optimal (sub opt)) and the (co)variance matrices from the RR model, and the difference between the two as a percentage

	Cows			Bulls		
DIM	opt	sub opt	Percentage	opt	sub opt	Percentage
1-56	0.17	0.16	93%	0.98	0.92	93%
56-105	0.22	0.21	96%	0.98	0.97	99%
105-154	0.25	0.25	100%	1.00	0.99	99%
154-203	0.25	0.25	99%	1.00	1.00	100%
203-252	0.23	0.23	100%	0.99	0.97	98%
252-301	0.23	0.22	99%	0.95	0.93	97%
301-350	0.18	0.17	95%	0.95	0.92	97%
350-399	0.13	0.12	94%	0.98	0.91	93%

Using shorter recording periods, of three weeks, also showed the higher value of mid lactation records (Table 4.6, see Appendix A1 for the full table). However, the estimated reliabilities for bulls decreased notably at the start and end of the lactation. When using the fixed regression repeatability model (Leg 0), while assuming the estimated (co)variances from the RR are reality, only 73% of the reliability was realised with measurements taken in the first three weeks of the lactations. Nonetheless, between 29 and 372 DIM the differences were small, and high reliabilities for bulls could be achieved with either model (0.97 – 0.98).

Table 4.6. The reliability of the selection index (r^2) with phenotypes only being available the first and last three weeks of lactation as sequential ranges of 21 days in milk (DIM), when using the phenotypic (co)variance matrix (Cows), and genetic (co)variance matrix as phenotypes (Bulls) for the random regression model of the fourth order (optimal (opt)), with using the selection index coefficients coming from the fixed regression repeatability model (sub optimal (sub opt)) and the (co)variance matrices from the RR model, and the difference between the two as a percentage (For the full table see Appendix A1)

	Cows			Bulls		
DIM	opt	sub opt	Percentage	opt	sub opt	Percentage
1-21	0.12	0.11	92%	0.95	0.69	73%
21-35	0.15	0.14	99%	0.97	0.91	93%
35-49	0.16	0.16	100%	0.98	0.98	100%
357-371	0.12	0.12	100%	0.97	0.97	100%
371-385	0.11	0.11	99%	0.95	0.89	93%
385-399	0.09	0.08	94%	0.90	0.65	72%

When looking at the cumulative trend of collecting weekly measurements over a lactation on the reliability, large differences can be observed in the increase in reliability at the start of the lactation (Figure 4.6). When using the Leg 0 model, the reliabilities will be inflated at the start of the lactation, both in the Cows and especially the Bulls scenarios (Figure 4.6). Predicting a reliability of one after one week of recording many daughters, whereas the true reliability is much lower. Also, it takes more weeks to achieve a true reliability of selection, when using the suboptimal fixed regression model versus the RR model (approximately 90 versus 30 weeks).

4.4 Discussion

The aim of this research was to study the heritability and repeatability of measured CH_4 concentrations over a lactation using a RR model, and to compare using the fixed regression repeatability model and a RR model for genetic evaluations under different phenotyping strategies. The data used was a novel data set from emissions measured on 4,664 cows from 52 farms located across the Netherlands, with up to 37 weeks of the lactation recorded, which has previously only been analysed using fixed regression repeatability models.

4.4.1 Order of polynomials for RR models

In this study we estimated genetic parameters, using different orders of Legendre polynomials in a RR model, ranging from the 1st to the 5th order. In our study, the models with higher order polynomials had better goodness of fit (Table 4.3). This is similar to what

has been shown in studies on random regressions for milk yield using test-day models (Li et al., 2020; Pool et al., 2000; van Der Werf et al., 1998). However, as these studies also highlight, fitting higher order polynomials comes at a higher computational cost. Since for the higher orders fitted the increase in goodness of fit becomes less, the common consensus between the previous studies is that fitting at least third order polynomials are considered sufficient when using heterogeneous residual variances. Furthermore, fitting higher order polynomials can induce oscillatory patterns along the lactation, which are unlikely to be biological (Pool et al., 2000). This highlights that more parsimonious models should be preferred when the improvement in model fit is limited. In the final results of this paper, the model fitted with fourth order Legendre polynomials was applied for the random additive genetic and within lactation permanent environmental effects, as the fifth order model resulted in large deviations in the heritability at the end of the lactation (Figure 4.2) and in large deviations in genetic correlations in the beginning and end of the lactation, which are difficult to explain (result not shown).

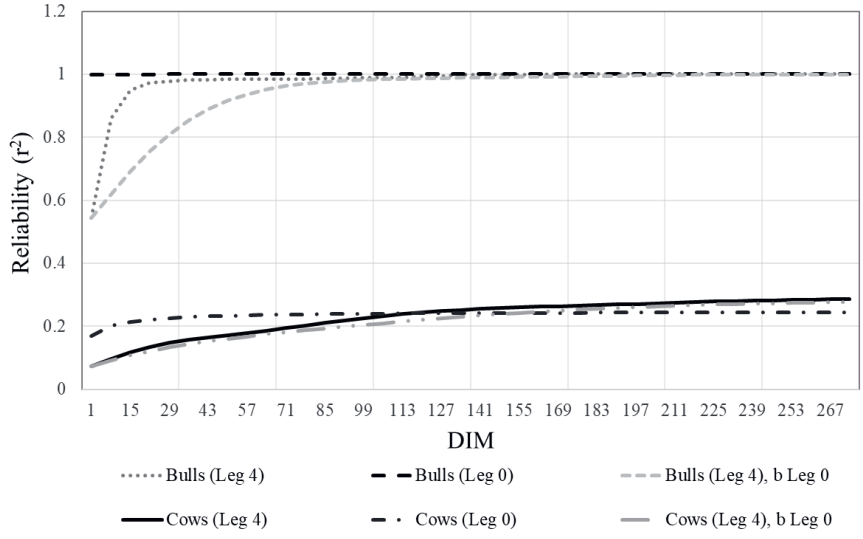

Figure 4.6. The reliability of the selection index (r^2) for the random regression model using Legendre polynomials of the fourth order (Leg 4), the fixed regression repeatability model (Leg 0), and with using the selection index coefficients coming from the fixed regression repeatability model (b Leg 0) and the (co)variance matrices from Leg 4, on Bulls or Cows, with phenotypes being available for a cumulative number of days in milk (DIM) ranging from only 1 DIM to 274 DIM

4.4.2 Heritability and repeatability within lactations

The heritability for weekly mean CH_4c increased steeply in early lactation, peaked at 134 DIM, whereafter it steadily decreased again (Figure 4.2, top). The average heritability was 0.17, with a low standard error of 0.04. The standard errors of the estimates are lower than of previous studies, giving more confidence in the estimates of this study (Breider et al., 2019; Manzanilla-Pech et al., 2022a; Pszczola et al., 2017). The pattern of the heritability within a lactation showed similarities to the study by Pszczola et al. (2017), who reported that the heritability for CH_4p (CH_4 production, estimated from sniffer CH_4c measurements) was highest in mid lactation. Although, the reported heritability estimates were less variable and ranged between 0.23 ± 0.12 and 0.3 ± 0.08. A similar pattern was also observed in the study by Manzanilla-Pech et al. (2022a), where the heritabilities for CH_4c ranged between 0.10 and 0.28. In a study by Breider et al. (2019), a different pattern was observed for the heritability of CH_4c over a lactation, compared to the afore mentioned studies. Breider et al. (2019) showed that the heritability was the lowest in mid lactation and increased towards the end of the lactation. The study by Manzanilla-Pech et al. (2022a) discussed that this was possibly an outcome of the inclusion of multiple lactations in the analyses by Breider et al. (2019), whereas the former study analysed first and second lactation separately. However, in this study multiple lactations were also combined. Another factor that could have influenced the heritability estimates was that Breider et al. (2019) applied two bivariate models, with CH_4c modelled with milk yield and body weight, respectively. Also, the study by Sypniewski et al. (2021) reported a different pattern of heritability over the lactation. There, the heritability for CH_4c increased throughout the full lactation, whereas the pattern for the heritability of CH_4p was similar to this study. In the study by Sypniewski et al. (2021) the residual was modelled with homogeneous variances, which may have resulted in a different partitioning of variance between the genetic and permanent environmental variance within the lactation compared to this study.

For all order models fitted, the within lactation repeatability was relatively stable throughout the lactation (at approximately 0.58, Figure 4.2, bottom). This indicates that the within animal environmental variance is similar for all measures taken throughout the lactation, and that there are no parts of the lactation that would benefit form a larger number of measurements than other parts. The within lactation repeatability reported in this study was of similar pattern, although slightly lower, than what was reported in the study by Manzanilla-Pech et al. (2022a) for CH_4c, where they ranged between 0.63 and 0.86. However, in the study by Manzanilla-Pech et al. (2022a) a separate across lactation permanent environment effect was not fitted. Including the across lactation permanent environmental effect, as the total repeatability, the repeatability would be 0.80 and thereby similar to what was reported previously. Other estimates for the repeatability of CH_4c within lactations have not been previously reported.

The increase in heritability at the start of the lactation was a result of lower additive genetic variance at the beginning of the lactation, which can be observed in Figure 4.3. However, caution should be taken in interpreting the first and last days of the lactation, as models fitted with polynomials are known to deviate at the far extremes (Pool et al., 2000). The additive genetic and the permanent environmental variance started to deviate before approximately 50 DIM and after 300 DIM (for the models fitted with second to fifth order Legendre polynomials). A similar pattern has been reported in studies on milk yield using RR models (Pool et al., 2000; Strabel et al., 2005; van Der Werf et al., 1998). Higher permanent environmental variances at the beginning and end of the lactation may be attributed to a lower number of records that often are available at the start and end of the lactation. It is therefore expected that the large increase in permanent environmental variance at the end of the lactation in this study was in part an artifact of the data. From Figure 4.1 (left) it can be observed that the number of records at the beginning and at the end of the lactation were low, with under 50 weekly mean CH_4c records per DIM after 300 DIM and decreasing. The deviations influenced the heritability before ten DIM and after 300 DIM, which is underlined by the higher associated standard errors at the beginning and towards the end of the lactation. The extent of the effect of a lower number of records at the beginning and end of the lactation should be further investigated, and could be overcome, for example, by fitting models using a smoothing factor such as splines (White et al., 1999).

4.4.3 Genetic and permanent environmental correlations within lactations

The genetic correlations between DIM were high, except between some DIM at the far ends of the lactation (Figure 4.5, left). High and positive genetic correlations indicate that, even for most records taken far apart in time, the direction of selecting for lower CH_4c would be the same. In addition, the magnitude of the genetic correlations has an influence on the reliability of the selection index of measurements taken along the lactation, which will be discussed later. The genetic correlations of weekly mean CH_4c between DIM were similar to what has been reported in previous studies (Manzanilla-Pech et al., 2022a; Pszczola et al., 2017). Nonetheless, there were some differences. The study by Manzanilla-Pech et al. (2022a) reported that the genetic correlations were higher and close to one along the full lactation in the second lactation, in contrast to the first lactation where the genetic correlations reduced for weeks that were the furthest apart in time. This should be investigated further, as it may implicate that a different phenotyping strategy should be maintained for first or later lactation cows. Nonetheless, the model in this study included a between parity random effect and a fixed effect for the lactation curve as an interaction with parity, to correct for differences between parities.

The permanent environmental correlations between weekly mean CH_4c measurements closely together in time were high, but the correlations reduced and became negative for

measurements further apart in time (Figure 4.5, right). This confirms that modelling CH_4 including a permanent environmental effect is the most appropriate, ideally with random regressions, as different lactation stages are associated with differences in the permanent environmental variance. Permanent environmental correlations have not been previously reported in the literature.

Similar to the heritability and repeatability, the genetic and permanent environmental correlations may have been influenced by the lower number of records at the start and towards the end of the lactation. Therefore, caution should be taken in interpreting the results before 10 DIM and after 300 DIM, and the results should be confirmed using a data set that includes a similar number of records throughout the lactation or should be modelled using splines.

4.4.4 Selection index calculations

The selection index coefficient is directly linked to the maximum expected response in the aggregate genotype, and the reliability that would be published next to the breeding values. Multiple scenarios were simulated, and each scenario was simulated in twofold: 1) using the phenotypic (co)variance matrices (Cows), and 2) replacing the phenotypic relationship matrix by the genetic (co)variance matrices (Bulls). The first represented scenarios where breeding values would be estimated for a cow with single measurements at each class of DIM, whereas the second represented scenarios where breeding values would be estimated for a bull with a large or infinite number of daughters with many records. In the second case it is expected that the phenotypic (co)variance matrix approximates the genetic (co)variance matrix, and thus breeding values would become 100% reliable. This was indeed the case when full lactations would be measured, as can be observed in Table 4.4.

The reliabilities for a cow with measurements of a full lactation were higher for the RR model with high orders. Especially, the fifth order RR model resulted in a large increase in the estimated reliability and was 0.43. Nonetheless, this high reliability should be interpreted with care, and should be confirmed with using a model that applies a smoothing factor such as splines, as the results for the fifth order RR model showed deviations from the other models. Therefore, in this study we focussed on the RR model using fourth order Legendre polynomials.

When using the sub-optimal selection index coefficients of the fixed regression repeatability model, with (co)variance estimates of a fourth order RR repeatability model, an almost identical reliability was reached as when using the optimal selection index coefficients of RR model (98% for cows and 100% for bulls, Table 4.4). That would suggest that there is not a large gain in using a RR model over a fixed regression repeatability model. Nonetheless, for the bulls scenario the sub-optimal fixed regression repeatability selection index coefficients always realised 100% of the reliability compared to the RR model, suggesting that for bulls with a large number of daughters recorded over the full lactation there is never an advantage

in using a RR model over a fixed regression repeatability model. Although this might not be a practical scenario, it reflects the loss in selection reliability, based on the genetic architecture of the trait. That is obviously small when the true breeding value is known at any moment in time, because the genetic correlations between the time periods play no role in the genetic evaluation.

Table 4.4. The reliability of the selection index (r^2) for models using different orders of Legendre polynomials (Leg) for random effects (optimal (opt)), when using the phenotypic (co)variance matrix (Cows), or genetic (co)variance matrix as phenotypes (Bulls), and the two scenario's while using the selection index coefficients coming from the fixed regression repeatability model (sub optimal (sub opt)), with the difference between the two as a percentage

	Cows			Bulls		
	opt	sub opt	Percentage	opt	sub opt	Percentage
Leg 0	0.24	0.24	100%	1.00	1.00	100%
Leg 1	0.27	0.24	92%	1.00	1.00	100%
Leg 2	0.29	0.28	96%	1.00	1.00	100%
Leg 3	0.29	0.27	95%	1.00	1.00	100%
Leg 4	0.29	0.28	98%	1.00	1.00	100%
Leg 5	0.43	0.31	71%	1.00	1.00	100%

When measurements are available over only a short period of time, the RR repeatability model will most likely perform better compared to a fixed regression repeatability model. To investigate the effect of shorter recording period, three simulations were performed, with: 1) sequential periods of eight weeks of recording, 2) sequential periods of three weeks of recording, and 3) starting at one DIM and cumulatively adding one week of recording up to 274 DIM. This is relevant for phenotyping strategies, with only limited logistic and/ or financial resources available, by which they are not able to record full lactations and is especially relevant in farming systems with seasonal calving. When recording short periods of the lactation, the realised reliability was lower than when a full lactation would be recorded, with increasingly lower reliabilities for shorter recording periods. The difference in reliabilities was smaller for records taken during mid lactation (Table 4.5 and 4.6). For short recording periods, from the data reported in this study it can be concluded that records taken between approximately 100 DIM and 200 DIM will most likely yield the highest reliability and thereby the highest genetic progress, regardless of the model chosen. Thus, a fixed regression repeatability model in this case can perform sufficient and might be the better choice when there is limited data available or limited computational power.

For short recording periods at the start of the lactation (1 to 100 DIM), the reliabilities of the fixed regression repeatability model were higher than the reliabilities of the RR model for the cows and bulls scenarios (Figure 4.6). Here, the fixed regression model likely overestimates the reliability, as this model assumes equal genetic correlations between DIM, whereas the RR model showed that the first DIM had lower genetic correlations with the rest of the lactation. This has important implications for practice, and the results suggest that when breeding values are estimated from a fixed regression model and are only recorded during the first ±50 DIM, the realised genetic gain may become lower than expected. Therefore, in these situations using a RR model should be preferred.

4.5 Conclusions

Knowledge of the covariance structure of CH_4 over lactation is crucial for genetic evaluations under many different phenotyping strategies. We found the highest heritability mid-lactation (0.17 ± 0.04) and high genetic correlations between lactation stages (0.34 ± 0.36 to 0.91 ± 0.08). Permanent environmental correlations varied widely (-0.73 ± 0.08 to 1.00 ± <0.01). While bull reliability was similar for full lactation records using fixed and RR models, the fixed model's reliability dropped to 72% for shorter periods recorded at the start and end of the lactation. Additionally, assuming the fixed model whereas the true (co)variance structure is reflected by the RR model, more than twice as long recording from the start of lactation was required to achieve maximum reliability for a bull. Thus, using a simplistic model could result in implementing too little recording, and lower genetic gains than predicted from the reliability.

Acknowledgements

This study was financially supported by the TKI Agri & Food project LWV19155 and the partners CRV (Arnhem, the Netherlands) and FrieslandCampina (Wageningen, the Netherlands), and was supported by the Climate Approach project funded by the Dutch ministry for agriculture, nature and food quality (LNV, the Hague, the Netherlands) (project number BO-53-003). The authors gratefully acknowledge the farmers for participating in this research, Theo van Hattum and Harm Wemmenhove (Wageningen Livestock Research, Wageningen, the Netherlands) for installing and maintaining the sniffers, Gerrit Seigers (IT Development, Wageningen, the Netherlands) for setting up the data platform for sniffers, Arthur Gilmour helping with the XFA option in ASREML, and CRV for providing the pedigree data and other cow information. The authors declare there were no conflicts of interest.

Appendix

A1. The reliability of the selection index (r2) with phenotypes only being available as sequential ranges of 21 days in milk (DIM) (three weeks), when using the phenotypic (co)variance matrix (Cows), and genetic (co)variance matrix as phenotypes (Bulls) for the random regression model of the fourth order (RR), with using the selection index coefficients coming from the repeatability model (b Leg 0) and the (co)variance matrices from the RR model, and the difference between the two as a percentage

	Scenarios cows			Scenarios bulls		
DIM	RR	b Leg 0	Percentage	RR	b Leg 0	Percentage
1-21	0.12	0.11	92%	0.95	0.69	73%
21-35	0.15	0.14	99%	0.97	0.91	93%
35-49	0.16	0.16	100%	0.98	0.98	100%
49-63	0.17	0.17	100%	0.98	0.98	100%
63-77	0.19	0.18	100%	0.97	0.97	100%
77-91	0.20	0.20	100%	0.97	0.97	100%
91-105	0.21	0.21	100%	0.97	0.97	100%
105-119	0.23	0.23	100%	0.98	0.97	100%
119-133	0.24	0.24	100%	0.98	0.98	100%
133-147	0.24	0.24	100%	0.99	0.99	100%
147-161	0.24	0.24	100%	1.00	1.00	100%
161-175	0.24	0.24	100%	1.00	1.00	100%
175-189	0.23	0.23	100%	1.00	1.00	100%
189-203	0.23	0.23	100%	0.99	0.99	100%
203-217	0.22	0.22	100%	0.99	0.98	100%
217-231	0.22	0.22	100%	0.98	0.97	100%
231-245	0.22	0.22	100%	0.96	0.96	100%
245-259	0.22	0.22	100%	0.95	0.95	100%
259-273	0.21	0.21	100%	0.94	0.93	100%
273-287	0.21	0.21	100%	0.92	0.92	100%
287-301	0.19	0.19	100%	0.91	0.91	100%
301-315	0.18	0.18	100%	0.90	0.90	100%
315-329	0.16	0.16	100%	0.91	0.91	100%
329-343	0.14	0.14	100%	0.94	0.93	99%
343-357	0.13	0.13	100%	0.96	0.96	99%
357-371	0.12	0.12	100%	0.97	0.97	100%
371-385	0.11	0.11	99%	0.95	0.89	93%
385-399	0.09	0.08	94%	0.90	0.65	72%

5

Genetic relationships among methane emissions from breath, dry matter intake, body weight, and milk production traits of Dutch dairy cows

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Submitted for review

Abstract

Reducing enteric methane (CH_4) emissions from dairy cattle is essential to mitigate the environmental footprint of dairy farming. This study aimed to investigate genetic correlations between CH_4 emissions and key production traits in Dutch dairy cows combing two novel datasets. Methane concentrations (CH_4c) and CO_2 concentrations were measured using nondispersive infrared sensors, called sniffers, that sampled from the feed bin of automatic milking systems. Data on CH_4 were collected on 7,139 cows from 68 commercial farms in the Netherlands between 2019 and 2023. On an additional set of experimental farms, phenotypic data on milk production traits, body weight, and dry matter intake were recorded on 9,550, 7,049, and 7,546 cows, respectively. Genetic parameters were estimated with bivariate models for first, second, and third and later parity cows separately using ASReml v4.2.1. The heritability for CH_4c ranged from 0.21 to 0.27, while the repeatability ranged from 0.65 to 0.73 across different parities. Genetic correlations between CH_4c and dry matter intake were weak and positive, ranging from 0.06 to 0.15 across parities. Similarly, genetic correlations between CH_4c and milk production traits were weak, ranging from -0.04 to 0.23, with the highest correlations being with fat yield and fat percentage, which ranged across parities from 0.12 to 0.23 and 0.14 to 0.21, respectively. In addition, genetic correlations between CH_4c and body weight were weak, ranging from -0.04 to 0.06. Strong genetic correlations were observed between CH_4c and CO_2 concentrations, ranging from 0.81 to 0.97. Furthermore, the genetic correlations between CH_4c in different parities were high (0.86 to 0.97). The weak genetic correlations between CH_4c and production traits suggest that it is feasible to select for lower CH_4c, while improving milk production and other economically important traits.

Key words: methane emissions, genetic correlations, dairy cows, sniffer

5.1 Introduction

Animal breeding is increasingly acknowledged as an effective strategy to mitigate the environmental footprint of dairy farming. A novel trait of interest to genetic improvement, due to the direct impact on carbon footprint, is enteric methane (CH_4) emission. Enteric CH_4 is mostly produced by fermentation of feed in the rumen of cattle, which is emitted into the air by breath and belching (Jackson et al., 2020). Mitigating CH_4 emissions is urgent, as CH_4 is a potent greenhouse gas with a global warming potential that is approximately 27 times greater than CO_2 over a 100-year lifespan (IPCC, 2021). In addition, CH_4 plays a role in the formation of ozone in the atmosphere, which can cause health issues in humans and animals and can damage crops and ecosystems (Monks et al., 2015; Tie et al., 1992).

In the last decade, several publications have highlighted that there is promise in using animal breeding to mitigate CH_4 emissions (Lassen and Difford, 2020; Manzanilla-Pech et al., 2021). Still, questions remain in regard to how to implement the new traits into new or existing breeding programmes that aim to mitigate the environmental footprint of dairy farms. Essential parameters that are required for informed dairy breeding are the genetic correlations between CH_4 and other breeding goal traits (de Haas et al., 2017). Genetic correlations are used to predict the consequences of selection, to investigate undesired responses in traits, and to develop informed breeding goal traits for methane.

In the literature, reported estimates of genetic correlations between CH_4 and other important breeding goal traits have a large range and are in some cases contradictory. Genetic correlations between dry matter intake (DMI) and CH_4 emissions for example ranged from a weak negative correlation of -0.09 ± 0.38 to a moderate positive correlation of 0.60 ± 0.13 (Difford et al., 2020; Manzanilla-Pech et al., 2021). Similarly, genetic correlations between body weight and CH_4 emissions ranged from -0.18 ± 0.08 to 0.65 ± 0.07 (Breider et al., 2019; Difford et al., 2020; Lassen and Lovendahl, 2016; Manzanilla-Pech et al., 2021). For milk yield, fat yield, and protein yield, estimates of the genetic correlation with CH_4 ranged from 0.16 ± 0.06 to 0.54 ± 0.26 for milk yield (Breider et al., 2019; Lopez-Paredes et al., 2020; Manzanilla-Pech et al., 2021; Pszczola et al., 2019; van Engelen et al., 2018), from -0.15 ± 0.48 to 0.37 ± 0.07 for fat yield, and from 0.07 ± 0.06 to 0.46 ± 0.32 for protein yield (Lassen and Lovendahl, 2016; Lopez-Paredes et al., 2020; Pszczola et al., 2019; van Engelen et al., 2018). Currently, most estimates are associated with large standard errors and the overall results are therefore inconclusive. The large differences in estimates of genetic correlations with CH_4 traits was recently emphasised in a meta-analysis, which also concluded that current estimates of genetic correlations reported in the literature show large heterogeneity making inference for breeding difficult (Hossein-Zadeh, 2022). To be able to make informed selection decisions in the future, more accurate estimate of genetic correlations are thus required.

Therefore, the aim of this study was to investigate the relationship between CH_4 emissions, DMI, body weight, and milk production traits of Dutch dairy cows using a novel dataset of enteric CH_4 concentrations recorded by sniffers in automated milking systems. The dataset contains CH_4 recorded on a large number of cows, including 2,084 first parity cows, 1,843 second parity cows, and 3,743 third and later parity cows. In addition, data on DMI, milk production traits, and body weight were available on 7,546, 9,550 and 7,049 cows, respectively. Using this data, we gave further insight into the genetic relationship between CH_4, DMI, body weight, and milk production traits, which is required to implement the mitigation of CH_4 emissions into national breeding goals for dairy cows in the Netherlands.

5.2 Materials and Methods

Mean CH_4 concentrations (CH_4c) were recorded on 68 commercial dairy farms in the Netherlands. Data were collected between 2019 and 2023 and previously described in van Breukelen et al. (2024). In short, CH_4c were measured by nondispersive infrared sensors called "sniffers" (WD-WUR v1.0 and v2.0, manufactured by Carltech BV), which were installed with a sampling tube leading into the feed bin of automated milking systems (AMS). Methane and carbon dioxide (CO_2) concentrations were measured every five seconds, filtered to exclude biologically improbable records, and then averaged per AMS visit including only the records from the first and up to the fifth minute of milking. Thereafter, the records were corrected for diurnal variation within farm using a linear mixed model. The farms with CH_4 recording by sniffers were mainly farms without data available on DMI, body weight (BW) and milk production traits, with the exception of one farm that also had DMI recording. In total, 261 cows had CH_4 and DMI measured, of which nine cows were recorded for both traits within the same week.

The CH_4 dataset was combined with a dataset with information on DMI, BW and milk production traits recorded during DMI experiments, which contributed through the genetic relationships to the estimation of the genetic correlations with CH_4. The data on DMI, BW, milk yield (MY), protein yield (PY), and fat yield (FY) were recorded on several farms in the Netherlands between 1990 and 2023 (Table 5.1). For this research paper, no animal experiments were carried out and data on DMI were collected for previous research projects. The records resulted from several experiments previously described in the literature by Beerda et al. (2007), Veerkamp et al. (2000), Zom et al. (2012), and van Knegsel et al. (2014). The experiments were performed at several locations in the Netherlands, for example at: Aver Heino; Bosma Zathe in Ureterp; Cranendonck in Soerendonk; 't Gen in Lelystad; Minderhoudhoeve in Swifterbant; Waiboerhoeve Dairy unit 2, 3 in Lelystad; Zegveld farm in Zegveld; Hoorn in Lelystad; New Waiboerhoeve in Lelystad; and most recently at Dairy Campus in Leeuwarden. In addition, data was available of routine DMI recording by CRV

on an additional three farms. All cows were housed indoors, fed mixed diets ad libitum, and were milked two to three times a day in a milking parlour or milked by AMS during the experiments. Dry matter intake was recorded using individual automated feed bins (RIC bins, Insentec B.V.). The interval of recording DMI varied between experiments and ranged from one to five times per week. Specific dietary composition and energy of the diets were not available and DMI was estimated based on the dry matter composition of the diets. Body weight of the cows was measured with weighing platforms three times per week or daily, depending on the location of the cows. Milk yield, FY and PY were defined as the total yield per day in kg.

Table 5.1. The number of records and number of recorded cows per trait before and after editing

Trait[1]	Number of records			Number of cows		
	Original data	Weekly means	After filtering	Original data	Weekly means	After filtering
CH_4c	882,632	74,569	71,572	9,274	7,139	6,901
DMI	727,458	210,865	206,491	7,547	7,546	7,399
MY	1,235,834	544,562	532,905	9,549	9,550	9,366
PY	454,187	450,822	440,570	9,542	9,542	9,358
FY	452,151	450,034	439,785	9,544	9,544	9,360
BW	698,325	195,361	192,440	7,049	7.049	6,885

[1]CH_4c = methane concentration; DMI = dry matter intake; MY = milk yield; PY = protein yield; FY = fat yield; BW = body weight

5.2.1 Data editing

The data was filtered to exclude outliers using several criteria. Dry matter intake records of less than 8 kg per day and more than 55 kg per day and BW records of less than 300 kg and more than 1,000 kg were set to missing. Outliers for MY, FY, and PY were set to missing if the record was less or more than three standard deviations from the overall mean MY, FY, or PY, respectively. After filtering, all records were averaged to weekly records for each trait per cow to homogenise the data. Weekly records for CH_4c were set to missing if they consisted of less than five AMS visits recorded during that week. Cows with missing information for any trait remained in the analyses for the other traits. The number of weekly records for each trait and the number of cows with weekly records are given in Table 5.1.

After averaging the records to weekly means, additional filtering criteria were applied to prepare the data for the genetic analyses. All records which were not labelled to have been part of a DMI experiment were labelled with the herd, year, and quarter of the year as experiment. For DMI, only experimental treatments for DMI were retained with records for at least five unique animals. Cows which were less than 50% Holstein were discarded and not included in the analyses and records recorded at 406 DIM and later were set to missing. In addition, records of first parity cows with an age of calving under 20 months were discarded. Thereafter, records of DMI and BW for cows with less than three weekly records in a lactation, and of CH_4c for cows with less than five weekly records in a lactation or less than four records per herd per week were set to missing. In addition, CH_4c records within groups of year and week of the year, with less than four records within a farm were set to missing. Information on the main traits in the final dataset is given in Table 5.1.

After homogenizing and filtering the data, three additional traits were computed. Fat percentage (F%) and protein percentage (P%) were calculated as the FY or PY, respectively, divided by the kilograms of milk coming from the corresponding milk record. Thereafter, energy corrected milk yield (ECM) was calculated, using the following formula (Sjaunja et al., 1991):

$$ECM = 0.25\ milk\ yield\ (kg) + 12.2\ fat\ yield\ (kg) + 7.7\ protein\ yield\ (kg)$$

Finally, three subsets of the dataset were made including only first, second, or third and later parity records for all traits. The dataset was split into three subsets, to allow for more parsimonious modelling without the need to include a within and an across lactation permanent environmental effect. The number of records, number of cows, and descriptive statistics per group of parity are given in Table 5.2.

5.2.2 Pedigree and genotype data

Pedigree and genotype information were made available by the cooperative cattle improvement organization CRV (Arnhem, the Netherlands). The pedigree was pruned to include only phenotyped animals and their ancestors and the final pruned pedigree comprised 106,641 animals. Some of the phenotyped cows were also genotyped using the Eurogenomics 10k chip. Imputation was routinely performed by CRV and resulted in a final number of 55,008 SNPs with information on 8,194 animals, which were used for the analyses. From the pedigree and genotype data, a combined genomic relationship matrix ($\mathbf{H}^{-1}$ matrix) was constructed following the method of Aguilar et al. (2010) and Christensen and Lund (2010) with the software calc_grm version b202212 (Calus and Vandenplas, 2022). The final $\mathbf{H}^{-1}$ matrix comprised all 106,641 animals that were included the pedigree.

5.2.3 Genetic analyses

Variance components were estimated using restricted maximum likelihood methods in ASReml v4.2.1 (Gilmour et al., 2015) with the $\mathbf{H}^{-1}$ relationship matrix using a single step procedure. Bivariate models were run between each combination of traits and as pairwise analyses between CH_4c from first, second, and third and later parity cows. The following bivariate model was used:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1 & 0\\ 0 & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Za}_1 & 0\\ 0 & \mathbf{Za}_2\end{bmatrix}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Zp}_1 & 0\\ 0 & \mathbf{Zp}_2\end{bmatrix}\begin{bmatrix}\mathbf{pe}_1\\ \mathbf{pe}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Ze}_1 & 0\\ 0 & \mathbf{Ze}_2\end{bmatrix}\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

where $\mathbf{y}_i$ is a vector containing repeated records on each trait; $\mathbf{b}_i$ is a vector with fixed effects for each trait, which were: an interaction between farm, year and week of measurement for the traits CH_4c and CO_2 concentrations (CO_2c) only, an interaction between farm and experimental treatment for all traits except CH_4c and CO_2c, a second order Legendre polynomial on age at calving in days, a third order Legendre polynomial on DIM, and an interaction between the second breed with a second order Legendre polynomial on the fraction of the second breed; $\mathbf{a}_i$ is a vector with additive genetic effects of the cows in $\mathbf{y}_i$; $\mathbf{pe}_i$ is a vector with permanent environmental effects of the cows in $\mathbf{y}_i$; and $\mathbf{e}_i$ is a vector with residuals (the analyses on the third and later parity subset included an interaction between parity and the permanent environmental effect). $\mathbf{X}_i$, $\mathbf{Za}_i$, $\mathbf{Zpe}_i$, and $\mathbf{Ze}_i$ are incidence matrices linking the records of $\mathbf{y}_i$ to the fixed effects, covariates, additive genetic, permanent environmental and residual effects, respectively. For the runs between DMI and CH_4c or CO_2c, the residual and permanent environmental covariances were fixed to zero, because of a limited number of cows with records on both a greenhouse gas trait and DMI (parity one had 15 cows with records on both traits, of which one had records for both traits in the same week; parity two had 28 cows with records on both traits of which 2 had records in the same week; and parity three and later had 163 cows with records on both traits of which 6 had records in the same week). The additive genetic, and permanent environmental effects for all traits were assumed normally distributed with a mean of zero, a variance of σ^2_{ji} for random effect *j* and trait *i*, and for the non-fixed analyses a covariance between two traits of σ_{j1j2}:

$$\begin{bmatrix}j_1\\ j_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\ 0\end{pmatrix}, \mathbf{I} \otimes \begin{pmatrix}\sigma^2_{j_1} & \sigma_{j_1 j_2}\\ \sigma_{j_1 j_2} & \sigma^2_{j_2}\end{pmatrix}\right]$$

The estimated heritability (h^2) and repeatability (t) were defined as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

$$t = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

where σ^2_a is the additive genetic variance, σ^2_{pe} is the permanent environmental variance, and σ^2_e is the residual variance.

The genetic (r_g) and phenotypic (r_p) correlations were defined as:

$$r_g = \frac{\sigma_{a1a2}}{\sigma_{a1}\sigma_{a2}}$$

$$r_p = \frac{\sigma_{p1p2}}{\sigma_{p1}\sigma_{p2}}$$

where σ_{ai} is the additive genetic standard deviation of trait one or two and σ_{a1a2} is the additive genetic covariance between trait one and two, and σ_{pi} is the phenotypic standard deviation of trait one or two and σ_{p1p2} is the phenotypic covariance between trait one and two.

5.3 Results

5.3.1 Descriptive statistics

The means of CH_4c, DMI, MY, and BW for first parity cows were 515 ppm, 19.7 kg/d, 27.4 kg/d, and 600 kg, respectively (Table 5.2). The coefficients of variation (CV) ranged between 10% and 51% and were highest for CH_4c.

Table 5.2. Descriptive statistics for each trait: the number of cows, number of records after editing, trait unit, mean, standard deviation (SD), minimum, maximum, and coefficient of variation (CV), per group of parity, including parity one, parity two, and parity three and later (3+)

Trait[1]	Parity	N cows	N records	Unit	Mean	SD	Min	Max	CV (%)
CH_4c	1	2,084	17,936		515	259	12	2,369	50
	2	1,843	15,265	ppm	557	282	7	2,095	51
	3+	3,743	38,364		592	305	5	2,087	51
CO_2c	1	385	2,369		4,168	1,685	7	9,483	40
	2	390	2,090	ppm	4,324	1,711	13	9,511	40
	3+	758	5,389		4,315	1,730	6	9,837	40
DMI	1	4,998	87,306		19.7	3.6	8	51	18
	2	3,500	54,660	kg/d	22.1	4.2	8	53	19
	3+	2,943	64,525		22.5	4.5	8	50	20
MY	1	8,891	179,469		27.4	7.1	1.0	59.1	26
	2	7,553	139,875	kg/d	31.6	9.6	1.0	59.4	31
	3+	5,776	213,539		32.1	10.3	0.9	59.4	32
ECM	1	8,889	139,295		27.5	6.0	3.1	56.4	22
	2	7,529	112,539	kg/d	32.4	8.6	3.8	60.3	27
	3+	5,760	186,581		33.6	9.5	3.2	59.3	28
PY	1	8,889	139,317		0.90	0.2	0.2	1.9	24
	2	7,539	112,996	kg/d	1.09	0.3	0.2	1.9	27
	3+	5,772	188,235		1.11	0.3	0.2	1.9	28
FY	1	8,889	139,328		1.13	0.3	0.1	2.5	22
	2	7,538	112,990	kg/d	1.34	0.4	0.1	2.5	27
	3+	5,767	187,445		1.40	0.4	0.1	2.5	29
P%	1	8,889	139,314		3.56	0.4	1.3	18.7	11
	2	7,539	112,850	%	3.61	0.4	0.4	12.4	12
	3+	5,768	187,801		3.55	0.4	1.0	14.6	12
F%	1	8,889	139,326		4.39	0.8	1.3	22.5	17
	2	7,536	112,778	%	4.47	0.8	0.6	14.5	17
	3+	5,763	186,934		4.50	0.7	0.5	18.6	17
BW	1	5,919	119,523		600	72	306	988	12
	2	4,532	91,817	kg	667	75	301	995	11
	3+	4,194	164,715		713	71	310	1,000	10

[1] CH_4c = methane concentration before standardisation; CO_2c = carbon dioxide concentration before standardisation; DMI = dry matter intake; MY = milk yield; ECM = energy corrected milk yield; PY = protein yield; FY = fat yield; P% = protein percentage; F% = fat percentage; BW = body weight

5.3.2 Heritability and repeatability

The estimated heritabilities averaged over the bivariate runs ranged from 0.21 ± 0.04 to 0.27 ± 0.03 for CH_4c, and from 0.23 ± 0.15 to 0.44 ± 0.15 for CO_2c (Table 5.3). For the other traits, the lowest heritability was estimated for DMI in second parity cows (0.21 ± 0.02) and the highest heritability was estimated for BW in third and later parity cows (0.79 ± 0.01). The repeatability estimates were moderate to high and ranged from 0.46 ± 0.01 for first parity P% to 0.93 ± <0.01 for third and later parity BW.

Table 5.3. The heritability (h^2), repeatability (t), additive genetic variance (σ_a^2), permanent environmental variance (σ_{pe}^2), and residual variance (σ_e^2) for each trait as the mean of all bivariate runs

Trait[1]	Parity	h^2	t	σ_a^2	σ_{pe}^2	σ_e^2
CH_4c	1	0.21 ± 0.04	0.65 ± 0.01	0.10 ± 0.02	0.19 ± 0.01	0.16 ± <0.01
	2	0.24 ± 0.04	0.70 ± 0.01	0.13 ± 0.03	0.24 ± 0.01	0.16 ± <0.01
	3+	0.27 ± 0.03	0.73 ± 0.01	0.17 ± 0.02	0.29 ± 0.01	0.17 ± <0.01
CO_2c	1	0.23 ± 0.15	0.54 ± 0.05	0.13 ± 0.10	0.18 ± 0.05	0.26 ± 0.01
	2	0.44 ± 0.15	0.71 ± 0.04	0.34 ± 0.16	0.22 ± 0.08	0.23 ± 0.01
	3+	0.31 ± 0.07	0.72 ± 0.02	0.25 ± 0.07	0.33 ± 0.03	0.22 ± <0.01
DMI	1	0.29 ± 0.02	0.69 ± 0.01	3.53 ± 0.29	4.92 ± 0.14	3.79 ± 0.02
	2	0.21 ± 0.02	0.65 ± 0.01	3.52 ± 0.45	7.52 ± 0.27	5.92 ± 0.04
	3+	0.22 ± 0.02	0.67 ± 0.01	4.35 ± 0.44	8.98 ± 0.25	6.51 ± 0.04
MY	1	0.43 ± 0.01	0.72 ± 0.01	17.23 ± 0.83	11.82 ± 0.27	11.31 ± 0.04
	2	0.33 ± 0.02	0.70 ± 0.01	19.99 ± 1.26	21.76 ± 0.53	18.03 ± 0.07
	3+	0.45 ± 0.01	0.69 ± 0.01	34.04 ± 1.36	17.76 ± 0.31	23.10 ± 0.07
ECM	1	0.36 ± 0.01	0.67 ± 0.01	12.22 ± 0.65	10.32 ± 0.24	11.20 ± 0.04
	2	0.30 ± 0.02	0.65 ± 0.01	3.52 ± 0.45	7.52 ± 0.27	5.92 ± 0.04
	3+	0.42 ± 0.01	0.68 ± 0.01	26.73 ± 1.13	16.36 ± 0.29	20.52 ± 0.07
PY	1	0.39 ± 0.01	0.69 ± 0.01	0.02 ± <0.01	0.01 ± <0.01	0.01 ± <0.01
`	2	0.33 ± 0.02	0.67 ± 0.01	0.02 ± <0.01	0.02 ± <0.01	0.02 ± <0.01
	3+	0.48 ± 0.01	0.70 ± 0.01	0.04 ± <0.01	0.02 ± <0.01	0.02 ± <0.01
FY	1	0.31 ± 0.01	0.58 ± 0.01	0.02 ± <0.01	0.02 ± <0.01	0.02 ± <0.01
	2	0.28 ± 0.01	0.58 ± 0.01	0.03 ± <0.01	0.03 ± <0.01	0.04 ± <0.01
	3+	0.39 ± 0.01	0.62 ± 0.01	0.05 ± <0.01	0.03 ± <0.01	0.05 ± <0.01
P%	1	0.35 ± 0.01	0.46 ± 0.01	0.05 ± <0.01	0.02 ± <0.01	0.07 ± <0.01
	2	0.31 ± 0.01	0.47 ± 0.01	0.05 ± <0.01	0.02 ± <0.01	0.08 ± <0.01
	3+	0.43 ± 0.01	0.51 ± 0.01	0.08 ± <0.01	0.01 ± <0.01	0.09 ± <0.01
F%	1	0.44 ± 0.01	0.54 ± 0.01	0.26 ± 0.01	0.05 ± <0.01	0.27± <0.01
	2	0.45 ± 0.01	0.57 ± 0.01	0.27 ± 0.01	0.07 ± <0.01	0.27 ± <0.01
	3+	0.55 ± 0.01	0.60 ± 0.01	0.40 ± 0.01	0.03 ± <0.01	0.29 ± <0.01
BW	1	0.39 ± 0.02	0.87 ± <0.01	1,477 ± 112	1,788 ± 48	493 ± 2
	2	0.37 ± 0.02	0.88 ± <0.01	1,775 ± 161	2,481 ± 77	583 ± 3
	3+	0.79 ± 0.01	0.93 ± <0.01	6,944 ± 252	1,187 ± 30	610 ± 2

[1]CH_4c = methane concentration; CO_2c = carbon dioxide concentration; DMI = dry matter intake; MY = milk yield; ECM = energy corrected milk yield; PY = protein yield; FY = fat yield; P% = protein percentage; F% = fat percentage; BW = body weight

5.3.3 Phenotypic and genetic correlations

The genetic correlations between CH_4c and other traits were positive (Table 5.4, 5.5, and 5.6), except with MY for first parity cows and with BW for first and second parity cows which had weak negative correlations (all were -0.04). The milk production traits had weak genetic correlations with CH_4c, with the strongest genetic correlations being between CH_4c, and FY and F% (0.12 ± 0.08 to 0.23 ± 0.10 and 0.14 ± 0.06 to 0.21 ± 0.08, resp.). The genetic correlations between CH_4c and DMI were also weak, ranging from 0.06 ± 0.10 to 0.15 ± 0.12.

The genetic correlation between CH_4c and CO_2c was strong (0.81 ± 0.10 to 0.97 ± 0.02, Table 5.4, 5.5, and 5.6). Similar to the results for CH_4c, all other genetic correlations between CO_2c and the breeding goal traits were weak. The genetic correlation between CO_2c and DMI ranged between 0.19 ± 0.23 and 0.29 ± 0.16, with the genetic correlation of 0.29 ± 0.16 in

parity three plus being the strongest correlation between CO_2c and a breeding goal trait. The genetic correlations between DMI and the production traits MY, PY, and FY were strong, and ranged from 0.62 ± 0.03 to 0.77 ± 0.03 over parities (Table 5.4, 5.5, and 5.6). The genetic correlation between DMI and BW was weak to moderate and was weakest at 0.25 ± 0.02 in parity one and strongest at 0.48 ± 0.04 in parity three and later.

Phenotypic correlations between CH_4c measured in different parities were weak to moderate and were 0.12 ± 0.03, 0.20 ± 0.03, and 0.51 ± 0.04 (Table 5.7). The genetic correlations were strong and were: 0.97 ± 0.15 between parity one and two, 0.96 ± 0.14 between parity two and three plus, and 0.86 ± 0.20 between the most distant parities.

5.4 Discussion

The objective of this study was to investigate the genetic relationships between enteric CH_4c and key breeding goal traits in Dutch dairy cows, including DMI, BW, and milk production traits, to inform breeding programmes aimed at mitigating methane emissions. The findings revealed generally weak genetic correlations between CH_4c and the studied traits, with some positive (although weak) associations, particularly with FY and F%. The weak genetic correlations between CH_4c and production traits suggest that it is feasible to select for lower CH_4c, while improving milk production and other economically important traits.

5.4.1 Descriptive statistics

The average CH_4c ranged from 515 to 592 ppm over parities, which is similar to what was reported by Sypniewski et al. (2021) (505 and 517 ppm, for two farms), but lower than what was reported by Huhtanen et al. (2015), Rey et al. (2019), and Saborío-Montero et al. (2019) (758 and 1,405, 1,268, and 853, respectively). Differences between the level of emissions that were recorded between studies can be expected based on the type of sniffer that was used, the dimensions of the feed bin the sniffer was installed into, and filtering of data before calculating a mean over a day or week. The CH_4c and CO_2c traits had a high CV, which was previously addressed in an earlier paper on part of the data (van Breukelen et al., 2022).

Table 5.4. Phenotypic (above diagonal) and genetic (below diagonal) correlations between traits[1] for first parity cows

	CH_4c	CO_2c	DMI	MY	ECM	PY	FY	P%	F%	BW
CH_4c		0.84 ± 0.01	0.02 ± 0.03	0.05 ± 0.10	0.05 ± 0.10	0.02 ± 0.10	0.06 ± 0.10	-0.25 ± 0.09	-0.04 ± 0.09	0.03 ± 0.04
CO_2c	0.81 ± 0.10		0.05 ± 0.06	0.04 ± 0.17	0.01 ± 0.17	<0.01 ± 0.17	0.01 ± 0.17	-0.20 ± 0.22	-0.04 ± 0.17	-0.09 ± 0.08
DMI	0.06 ± 0.10	0.19 ± 0.23		0.50 ± 0.01	0.52 ± 0.01	0.54 ± 0.01	0.43 ± 0.01	0.06 ± 0.01	-0.16 ± 0.01	0.21 ± 0.02
MY	-0.04 ± 0.08	0.04 ± 0.16	0.62 ± 0.03		0.91 ± <0.01	0.92 ± <0.01	0.75 ± <0.01	-0.30 ± 0.01	-0.49 ± 0.01	0.10 ± 0.02
ECM	0.04 ± 0.08	0.07 ± 0.16	0.66 ± 0.03	0.92 ± 0.01		0.94 ± <0.01	0.94 ± <0.01	-0.04 ± 0.01	-0.12 ± 0.01	0.16 ± 0.02
PY	<0.01 ± 0.08	0.02 ± 0.16	0.66 ± 0.03	0.93 ± <0.01	0.96 ± <0.01		0.80 ± <0.01	0.08 ± 0.01	-0.30 ± 0.01	0.17 ± 0.02
FY	0.12 ± 0.08	0.13 ± 0.16	0.62 ± 0.03	0.73 ± 0.02	0.93 ± <0.01	0.81 ± 0.01		0.04 ± 0.01	0.18 ± 0.01	0.16 ± 0.01
P%	0.10 ± 0.09	-0.14 ± 0.23	0.11 ± 0.05	-0.40 ± 0.03	-0.10 ± 0.03	-0.02 ± 0.03	0.06 ± 0.04		0.54 ± 0.01	0.14 ± 0.01
F%	0.21 ± 0.08	0.23 ± 0.25	-0.19 ± 0.04	-0.63 ± 0.02	-0.28 ± 0.03	-0.42 ± 0.03	0.08 ± 0.03	0.65 ± 0.02		0.03 ± 0.02
BW	-0.04 ± 0.10	-0.04 ± 0.26	0.25 ± 0.02	0.15 ± 0.04	0.19 ± 0.04	0.21 ± 0.04	0.19 ± 0.05	0.16 ± 0.04	-0.06 ± 0.04	

[1]CH_4c = methane concentration; CO_2c = carbon dioxide concentration; DMI = dry matter intake; MY = milk yield; ECM = energy corrected milk yield; PY = protein y ield; FY = fat yield; P% = protein percentage; F% = fat percentage; BW = body weight

Table 5.5. Phenotypic (above diagonal) and genetic (below diagonal) correlations between traits[1] for second parity cows

	CH_4c	CO_2c	DMI	MY	ECM	PY	FY	P%	F%	BW
CH_4c		0.89 ± 0.01	0.04 ± 0.03	0.02 ± 0.08	0.08 ± 0.07	0.05 ± 0.08	0.11 ± 0.07	0.08 ± 0.07	0.12 ± 0.06	-0.01 ± 0.05
CO_2c	0.93 ± 0.05		0.07 ± 0.07	0.06 ± 0.12	0.08 ± 0.12	0.05 ± 0.12	0.07 ± 0.11	-0.01 ± 0.12	-0.03 ± 0.12	0.03 ± 0.09
DMI	0.15 ± 0.12	0.22 ± 0.21		0.45 ± 0.01	0.49 ± 0.01	0.48 ± 0.01	0.42 ± 0.01	-0.02 ± 0.01	-0.11 ± 0.02	0.22 ± 0.02
MY	0.05 ± 0.09	0.13 ± 0.15	0.64 ± 0.05		0.91 ± <0.01	0.91 ± <0.01	0.74 ± 0.01	-0.38 ± 0.01	-0.45 ± 0.01	0.04 ± 0.02
ECM	0.14 ± 0.09	0.15 ± 0.15	0.70 ± 0.05	0.88 ± 0.01		0.94 ± <0.01	0.95 ± <0.01	-0.09 ± 0.01	-0.05 ± 0.01	0.09 ± 0.02
PY	0.08 ± 0.01	0.10 ± 0.14	0.66 ± 0.05	0.92 ± 0.01	0.96 ± <0.01		0.80 ± <0.01	0.02 ± 0.01	-0.23 ± 0.01	0.12 ± 0.02
FY	0.23 ± 0.10	0.18 ± 0.16	0.66 ± 0.05	0.66 ± 0.02	0.93 ± 0.01	0.81 ± 0.01		<0.01 ± 0.01	0.24 ± 0.01	0.07 ± 0.02
P%	0.12 ± 0.09	-0.13 ± 0.19	-0.04 ± 0.06	-0.36 ± 0.04	0.02 ± 0.04	0.04 ± 0.04	0.23 ± 0.04		0.58 ± 0.01	0.15 ± 0.02
F%	0.20 ± 0.08	0.05 ± 0.18	-0.14 ± 0.06	-0.55 ± 0.03	-0.08 ± 0.04	-0.26 ± 0.03	0.28 ± 0.04	0.73 ± 0.02		0.03 ± 0.02
BW	-0.04 ± 0.11	0.04 ± 0.20	0.28 ± 0.07	0.22 ± 0.05	0.27 ± 0.05	0.29 ± 0.05	0.24 ± 0.06	0.15 ± 0.05	-0.04 ± 0.05	

[1]CH_4c = methane concentration; CO_2c = carbon dioxide concentration; DMI = dry matter intake; MY = milk yield; ECM = energy corrected milk yield; PY = protein yield; FY = fat yield; P% = protein percentage; F% = fat percentage; BW = body weight

Table 5.6. Phenotypic (above diagonal) and genetic (below diagonal) correlations between traits[1] for third and later parity cows

	CH_4c	CO_2c	DMI	MY	ECM	PY	FY	P%	F%	BW
CH_4c		0.89 ± 0.01	0.02 ± 0.02	0.07 ± 0.04	0.12 ± 0.04	0.08 ± 0.04	0.13 ± 0.04	0.02 ± 0.03	0.06 ± 0.03	0.06 ± 0.03
CO_2c	0.97 ± 0.02		0.07 ± 0.04	0.07 ± 0.06	0.08 ± 0.06	0.08 ± 0.06	0.08 ± 0.06	0.01 ± 0.06	0.01 ± 0.06	0.15 ± 0.06
DMI	0.09 ± 0.10	0.29 ± 0.16		0.52 ± 0.01	0.51 ± 0.01	0.53 ± 0.01	0.43 ± 0.01	-0.01 ± 0.01	-0.11 ± 0.02	0.27 ± 0.02
MY	0.03 ± 0.06	0.12 ± 0.13	0.72 ± 0.03		0.91 ± <0.01	0.91 ± <0.01	0.74 ± <0.01	-0.34 ± 0.01	-0.45 ± 0.01	0.06 ± 0.02
ECM	0.08 ± 0.07	0.17 ± 0.13	0.78 ± 0.03	0.88 ± 0.01		0.94 ± <0.01	0.95 ± <0.01	-0.04 ± 0.01	-0.04 ± 0.01	0.12 ± 0.02
PY	0.03 ± 0.06	0.16 ± 0.12	0.75 ± 0.03	0.91 ± <0.01	0.96 ± <0.01		0.81 ± <0.01	0.08 ± 0.01	-0.22 ± 0.01	0.15 ± 0.02
FY	0.15 ± 0.07	0.22 ± 0.13	0.77 ± 0.03	0.68 ± 0.01	0.93 ± <0.01	0.83 ± 0.01		0.04 ± 0.01	0.24 ± 0.01	0.12 ± 0.02
P%	0.03 ± 0.06	0.22 ± 0.13	0.03 ± 0.05	-0.30 ± 0.02	0.10 ± 0.03	0.14 ± 0.02	0.27 ± 0.03		0.57 ± 0.01	0.14 ± 0.02
F%	0.14 ± 0.06	0.13 ± 0.13	-0.08 ± 0.05	-0.54 ± 0.02	-0.10 ± 0.02	-0.26 ± 0.02	0.24 ± 0.02	0.72 ± 0.01		0.06 ± 0.02
BW	0.06 ± 0.06	0.21 ± 0.12	0.48 ± 0.04	0.09 ± 0.03	0.15 ± 0.03	0.17 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.06 ± 0.03	

[1]CH_4c = methane concentration; CO_2c = carbon dioxide concentration; DMI = dry matter intake; MY = milk yield; ECM = energy corrected milk yield; PY = protein yield; FY = fat yield; P% = protein percentage; F% = fat percentage; BW = body weight

The minimum CH_4c was 5 ppm and the minimum CO_2c was 6 ppm, which is lower than what would be expected from the biology of the cow. The low minima can be explained from drifting of sensor calibration towards zero. Moments with extreme drift were filtered out of the dataset, although this was not sufficient to prevent the occurrence of low records after subtracting background concentrations and correcting for diurnal variation. As the CH_4 and CO_2 records around the minima were not detected as outliers in the dataset, these records were not filtered out of the data and are not expected to have had an effect on the genetic parameter estimates. For first parity cows, the average MY was 27.4 kg/d, the average BW was 600 kg, and the average DMI was 19.7 kg/d (Table 5.2), which is similar to previous estimates on first parity cows (Manzanilla-Pech et al., 2022a). For all three traits, the averages increased for second, third and later parity cows, as would be expected from the biology of the cow. The heritability and repeatability estimates for CH_4c were similar to what was previously estimated and discussed in a study using the same data (van Breukelen et al., 2024), however were slightly higher for parity two (0.24 ± 0.04 and 0.70 ± 0.01, resp.) and third and later parities (0.27 ± 0.03 and 0.73 ± 0.01, resp.). Heritabilities for MY, FY and PY were within the ranges of estimates used for genetic evaluations, which includes data from the Netherlands (Miglior et al., 2017).

Table 5.7. Phenotypic (above diagonal) and genetic (below diagonal) correlations between methane concentrations in parity one (Par1), parity two (Par2), and parity three including later parities (Par3+)

	Par1	Par2	Par3+
Par1		0.51 ± 0.04	0.12 ± 0.03
Par2	0.97 ± 0.15		0.20 ± 0.03
Par3+	0.86 ± 0.20	0.96 ± 0.14	

The genetic relationships between milk production traits, BW, and DMI have been extensively addressed in the literature (Berry et al., 2003; Calus and Veerkamp, 2011; Harris et al., 1992; Veerkamp and Thompson, 1999; Veerkamp et al., 2000; Veerkamp et al., 2001; Visscher and Goddard, 1995). However, relatively few studies have investigated the genetic relationships of these traits with CH_4 emissions and estimates are often associated with large SE. Therefore, the remainder of the discussion focusses on the relationships of the studied breeding goal traits with CH_4 emissions in dairy cows. The animals included in the study were an accurate representation of the Dutch national dairy herd, therefore, the genetic parameters estimated could apply in new national genetic evaluations for CH_4c.

5.4.2 Genetic correlation with dry matter intake

The phenotypic and genetic correlations between CH_4c and milk production traits, DMI, and BW estimated in this study were weak and ranged from -0.25 ± 0.09 to 0.23 ± 0.10 over parities (Table 5.4, 5.5, and 5.6). The genetic correlations between CH_4c and DMI estimated in this study were 0.06 ± 0.10 for first parity cows, 0.15 ± 0.12 for second parity cows, and 0.09 ± 0.10 for third and later parity cows. A previous study that estimated genetic correlations between CH_4c and DMI, using datasets in the Netherlands and Denmark, reported correlations of -0.09 ± 0.38 and 0.60 ± 0.13, respectively (Difford et al., 2020). The genetic correlation of -0.09 ± 0.38 was estimated from a dataset in the Netherlands, using a subset of the DMI data that was used for this study and was associated with a high SE. The estimates from this study indicate that a weak positive correlation between CH_4c and DMI is more likely.

A study by Manzanilla-Pech et al. (2021) estimated a genetic correlation between CH_4 production (estimated from sniffer concentration measurements using the formula as described by Madsen et al. (2010), and from here-on referred to as sniffer CH_4 production) and DMI of 0.42 ± 0.10. Similarly, a study that recorded CH_4 emissions using the SF_6 tracer method reported a moderate genetic correlation with DMI of 0.42 ± 0.30 (Richardson et al., 2021b). In addition, a study that used GreenFeed units to measure CH_4 production, averaged over seven days on 451 cows, reported a strong genetic correlation with DMI of 0.83 ± 0.11 (Lopes et al., 2024). Similar to sniffers, GreenFeed units measure spot samples of methane emissions from breath in a feed bin (Zimmerman, 2011). However, GreenFeed units also record head positioning and use an air flux method to measure total breath CH_4 emissions, which can extrapolated to total production in g CH_4/day using mass flux calculations (Huhtanen et al., 2015). Thereby, measurements by GreenFeed units are expected to provide a reliable estimate of true CH_4 production (Hristov et al., 2018; Velazco et al., 2016). Thus, although the genetic correlations between sniffer CH_4c and DMI were weak, the relationship between DMI and CH_4 production should be further investigated.

Measurements on CH_4c by sniffers can be used as an indicator for CH_4 production and the genetic correlation between sniffer CH_4c and GreenFeed CH_4 production has been estimated at 0.76 ± 0.15 (van Breukelen et al., 2023). In theory, a strong correlation between CH_4c and CH_4 production makes it unlikely that DMI is genetically correlated with CH_4 production but not with sniffer CH_4 concentrations. Therefore, the eigenvalues derived from the lowest estimated genetic correlation for first parity cows between CH_4c and DMI reported in this study were investigated. Because some eigenvalues were negative, a positive definite matrix was constructed for the matrix with the three genetic correlations. The genetic correlations, recomputed from the positive definite matrix, show that it is numerically not unlikely to have a strong genetic correlation between sniffer CH_4c and GreenFeed CH_4 production and strong genetic correlation between GreenFeed CH_4 production and DMI, while having a weak

genetic correlation between sniffer CH_4c and DMI (Table 5.8). Nonetheless, biologically the sniffer and GF phenotypes are expected to have similar genetic correlations with DMI and, therefore, the relationship between sniffer CH_4c and DMI should be further investigated.

Table 5.8. Approximated expected genetic correlations between sniffer CH_4 concentration (CH_4c, ppm), GreenFeed CH_4 production (GF CH_4p, g CH_4/day), and dry matter intake (DMI)

	Sniffer CH_4c	GF CH_4p
GF CH_4p	0.71	
DMI	0.08	0.78

5.4.3 Genetic correlations with milk yield

The genetic correlations between CH_4c and milk production traits reported in this study were weak. The genetic correlations with MY were -0.04 ± 0.08 for first parity cows, 0.05 ± 0.09 for second parity cows, and 0.03 ± 0.06 for third and later parity cows. These genetic correlations are lower than genetic correlations reported in previous studies which recorded CH_4 using sniffers. Studies by van Engelen (2018) and Lopez-Paredes et al. (2020) analysed CH_4c (ppm) and reported correlations with MY of 0.32 ± 0.06 and 0.17 ± 0.39, respectively. Other studies estimated sniffer CH_4 production and reported genetic correlations with MY ranging from 0.16 ± 0.06 to 0.54 ± 0.26 (Breider et al., 2019; Lopez-Paredes et al., 2020; Manzanilla-Pech et al., 2021; Pszczola et al., 2019). From the genetic correlations reported in the literature a positive relationship between MY and CH_4c was expected, however, the results from this study show that the relationship is likely very weak.

The estimated genetic correlations between sniffer CH_4 production and MY may be stronger than expected due to possible bias in the estimates of sniffer CH_4 production. Generally, sniffer CH_4 production is predicted using information on milk production (Madsen et al., 2010). Thus, moderate genetic correlations between sniffer CH_4 production and MY may be inflated by the sniffer CH_4 production phenotype also reflecting differences in MY.

5.4.4 Genetic correlations with fat and protein yield

The in this study estimated genetic correlations between CH_4c and FY were 0.12 ± 0.08, 0.23 ± 0.10, and 0.15 ± 0.07 for first, second, and third and later parity cows, respectively. Previous studies reported stronger, although still weak, genetic correlations with CH_4 concentrations of 0.37 ± 0.07 (van Engelen et al., 2018) and 0.27 ± 0.28 (Lopez-Paredes et al., 2020). For sniffer CH_4 production, studies by Pszczola et al. (2019) and Lopez-Paredes et al. (2020) reported positive genetic correlations with FY (0.21 ± 0.06 and 0.29 ± 0.28,

respectively), whereas a study by Lassen and Lovendahl (2016) reported a negative correlation although with a large SE (-0.15 ± 0.48). A study that used GreenFeed units to measure CH_4 production reported a strong estimate of the genetic correlation with FY of 0.89 ± 0.12 (Lopes et al., 2024). Thus, the genetic relationship between CH_4 production and FY is most likely positive, indicating that cows with a higher FY generally have a higher CH_4 production. However, the genetic correlation with sniffer measured CH_4c is weak and, therefore, selecting for lower CH_4c is not expected to have a large effect on FY.

The genetic correlations between CH_4c and PY were very weak and ranged from <0.01 ± 0.08 to 0.08 ± 0.01. Two previous studies reported stronger, although also weak, genetic correlations with CH_4 concentrations of 0.34 ± 0.06 (van Engelen et al., 2018) and 0.22 ± 0.41 (Lopez-Paredes et al., 2020). For sniffer CH_4 production, the correlation with PY was reported to be weak to moderate, ranging from 0.07 ± 0.06 to 0.46 ± 0.32 (Lassen and Lovendahl, 2016; Pszczola et al., 2019; van Engelen, 2018). In addition, the study that used GreenFeed units to measure CH_4 production reported a moderate genetic correlation with PY of 0.55 ± 0.14 (Lopes et al., 2024). Therefore, from the results of this study an effect of selecting for lower CH_4c on PY is not expected. However, it is still recommended to monitor FY and PY to ensure that undesired responses do not occur, as from the literature the genetic correlations with CH_4 production recorded by GreenFeed units appear to be stronger.

5.4.5 Genetic correlations with fat and protein percentage

Genetic correlations between CH_4c and F% were similar to the genetic correlations between CH_4c and FY and were 0.21 ± 0.08 for first parity cows, 0.20 ± 0.08 for second parity cows, and 0.14 ± 0.06 for third and later parity cows. In the literature, negative correlations have been reported of -0.05 ± 0.06 and -0.15 ± 0.48 (Lassen and Lovendahl, 2016; van Engelen, 2018), as well as positive correlations of 0.02 ± 0.40 and 0.37 ± 0.49 (Lassen and Lovendahl, 2016; Lopez-Paredes et al., 2020). Thus, the in the literature reported estimates have a large heterogeneity and are all not significantly different from zero, but the in this study estimated genetic correlations of 0.21 ± 0.08, 0.20 ± 0.08, and 0.14 ± 0.06 show that the genetic relationship between F% and sniffer recorded CH_4c is likely positive albeit weak. Similarly, estimates of the genetic correlation between F% and sniffer CH_4 production reported in the literature were weak and positive and were 0.04 ± 0.06 and 0.27 ± 0.36 (Lopez-Paredes et al., 2020; Pszczola et al., 2019). Therefore, a large effect of selecting for lower CH_4c on F% is not expected.

The estimates of the genetic correlation between CH_4c and P% were, similar to the correlations with PY, very weak and ranged from 0.03 ± 0.06 to 0.12 ± 0.09. Similar estimates have been reported in the literature of -0.06 ± 0.06 and 0.08 ± 0.32 (Lopez-Paredes et al., 2020; van Engelen, 2018). For sniffer CH_4 production, the in the literature reported correlations with P% were all positive. However, these estimates have large differences in

magnitude and ranged from 0.07 ± 0.06 to 0.77 ± 0.35 (Lassen and Lovendahl, 2016; Lopez-Paredes et al., 2020; Pszczola et al., 2019). Similar to MY, the genetic correlations between sniffer CH_4 production and P% may be stronger than expected, due to bias in converting sniffer CH_4 concentration to CH_4 production. The studies that reported strong correlations between sniffer CH_4 production and P%, calculated sniffer CH_4 production using the formula by Madsen et al. (2010) using fat and protein corrected milk. Thereby, sniffer CH_4 production may also reflect differences in PY and MY, resulting in a stronger genetic correlation with P%. Nonetheless, the genetic correlation with CH_4c is likely weak and therefore a large effect of selecting for lower CH_4c on P% is not expected.

5.4.6 Genetic correlations with energy corrected milk yield

The genetic correlations between CH_4c and ECM estimated in this study were weak (0.04 ± 0.08 to 0.14 ± 0.09 across parities). Previous studies reported a somewhat stronger genetic correlation between ECM and CH_4c of 0.35 ± 0.14 (Manzanilla-Pech et al., 2022a), and a moderate correlation of 0.45 ± 0.10 (Manzanilla-Pech et al., 2021) and strong correlation of 0.79 ± 0.05 (Manzanilla-Pech et al., 2022a) with sniffer CH_4 production. A study that recorded CH_4 emissions using the SF_6 tracer gas method reported a negative and weak genetic correlation of -0.08 ± 0.39, however, the estimate is associated with a large standard error (Richardson et al., 2021b). A study that used GreenFeed units to measure CH_4 production reported a weak genetic correlation with MY of 0.33 ± 0.12 and a strong genetic correlation with ECM of 0.74 ± 0.13 (Lopes et al., 2024). The stronger genetic correlation of CH_4c with ECM than with MY can be expected, due to the added information on FY in calculating ECM, which is more strongly correlated to CH_4 production. The reported genetic correlations of CH_4c with FY are somewhat stronger than with MY, thereby possibly contributing to the larger genetic correlations with ECM than with MY.

5.4.7 Genetic correlations using predicted methane

In the literature, additional estimates of genetic correlations have been reported between predicted CH_4 (predicted from milk production traits, DMI, BW, milk fatty acid composition and/ or milk mid-infrared spectrometry) and milk production traits. For example, genetic correlations between predicted CH_4 and MY ranged from -0.24 ± 0.30 to 0.89 (Bittante and Cecchinato, 2019; Ghiasi et al., 2022; Kandel et al., 2017; Pickering et al., 2015; van Engelen, 2018; Yin et al., 2015). This range is larger than the range for genetic correlations between recorded CH_4 and MY as was discussed above (-0.04 ± 0.08 to 0.54 ± 0.26). Predicted CH_4 emissions provide potential to build large databases for low additional costs, but the accuracy of prediction depends greatly on the prediction model that is used (Niu et al., 2018; Shadpour et al., 2022; van Gastelen et al., 2018). Therefore, prediction models should first be benchmarked through genetic correlations with recorded CH_4 emissions, for example, recorded by respiration chambers, GreenFeed units, sniffers, or other methods. This will help

to understand the genetic relationship between the various CH_4 predictor traits and other breeding goal traits. Weak genetic correlations between predicted CH_4 emissions and true CH_4 emissions may otherwise result in unexpected correlations with other traits (van Engelen, 2018), which, next to the high SE of the estimates, could partly explain the large heterogeneity in the estimates of genetic correlations reported in the literature and described in the meta-analysis by Hossein-Zadeh (2022).

5.4.8 Genetic correlation with body weight

The estimated genetic correlations between CH_4c and BW were very weak. The genetic correlation was -0.04 ± 0.10 for first parity cows, -0.04 ± 0.11 for second parity cows, and 0.06 ± 0.06 for third and later parity cows. A previous study, that estimated correlations between CH_4c and BW using two datasets, reported somewhat higher estimates of 0.16 ± 0.25 and 0.34 ± 0.16 (Difford et al., 2020). Previous estimates of the correlation between sniffer CH_4 production and BW showed a large heterogeneity, and the estimates ranged from a negative correlation of -0.18 ± 0.08 to a strong and positive correlation of 0.65 ± 0.07 (Breider et al., 2019; Lassen and Lovendahl, 2016; Manzanilla-Pech et al., 2021). Again, the genetic correlations between sniffer CH_4 production and BW may be inflated when BW is included in calculating sniffer CH_4 production as is done by the formula by Madsen et al. (2010). However, also the study that used GreenFeed units to measure CH_4 production reported a strong genetic correlation with metabolic BW of 0.68 ± 0.10 (calculated as $BW^{0.75}$, Lopes et al. (2024)). Thus, from the results of this study it appears that sniffer CH_4c are not correlated to BW. However, as other studies have reported the stronger correlation estimates with CH_4 production, the relationship should be monitored to avoid unexpected responses in BW when selecting for lower CH_4 emissions.

In the literature, genetic correlations between BW and MY have been shown to be close to zero, however, correlations between MY and body condition score (BCS) and with BW corrected for BCS were positive (Berry et al., 2003; Veerkamp and Brotherstone, 1997). Body condition score is an important indicator of the cow's predisposition to sustain high milk yields while, for example, going through a negative energy balance during early lactation. Previous genetic correlations between sniffer CH_4 production and BCS were estimated at -0.28 ± 0.10 (Zetouni et al., 2018) and 0.11 ± 0.10 (Manzanilla-Pech et al., 2021) and this indicates that in the case of CH_4 production the genetic correlation with BCS is also likely to be weak. Nonetheless, estimates between BCS and sniffer CH_4c have to date not been published and this genetic relationship should be evaluated in future studies.

5.4.9 Genetic correlation with carbon dioxide

Next to CH_4c, sniffers also record CO_2c. Although this greenhouse gas has received less attention in the literature to be used in genetic selection, CO_2c can potentially be applicable as an indicator for other traits in animal breeding practices (Difford et al., 2020). Previous

studies have shown that CO_2 production recorded using GreenFeed units is phenotypically correlated to DMI (0.36), feeding frequency (0.26), and feeding duration (0.18) in Holstein bulls (Callegaro et al., 2022). Similarly, a study using respiration chambers on Holstein cows reported even stronger phenotypic correlations between CO_2 production and DMI (0.93 ± <0.01) and CO_2 production and ECM (0.82 ± <0.01) (Huhtanen et al., 2021). Furthermore, CO_2c is a useful parameter that is applied in formulas to estimate CH_4 production from sniffer measurements (Madsen et al., 2010; Suzuki et al., 2021) and to estimate residual feed intake (RFI) (Huhtanen et al., 2021).

The in this study estimated genetic correlations between CO_2c and milk production traits, DMI, and BW were weak. A study by Difford et al. (2020) reported positive genetic correlations between sniffer CO_2c and BW of 0.19 ± 0.17 and 0.10 ± 0.17. These estimates are higher than the estimates reported in this study for first and second parity cows (-0.04 ± 0.26 and 0.04 ± 0.20, respectively), however, similar to the estimate for third and later parity cows (0.21 ± 0.12). This indicates that there may be a positive, but weak, genetic correlation between CO_2c and BW. The strongest genetic correlations over parities estimated in this study were between CO_2c and DMI, which ranged from 0.19 ± 0.23 to 0.29 ± 0.16. Estimates of the genetic correlations in the study by Difford et al. (2020) of CO_2c with DMI and RFI ranged from negative to positive (-0.08 ± 0.37 and 0.42 ± 0.13 for DMI, and -0.62 ± 0.38 to 0.54 ± 0.19 for various RFI traits). The estimate of -0.08 between CO_2c and DMI was estimated on a subset of the DMI records used for this study. Thus, from the results of this study it seems more likely that the genetic correlation between CO_2c and DMI is positive, although weak. However, the genetic correlation should be re-evaluated on a larger dataset, because the number of CO_2c records was small, with 2,369 records on 385 first parity cows, and SE were large.

In addition, the phenotypic and genetic correlations between CH_4c and CO_2c estimated in this study were strong (0.81 ± 0.01 and 0.97 ± 0.02, resp.). This is in agreement with a study that used respiration chambers and reported phenotypic correlations up to 0.96 between CH_4 production and CO_2 production (Aubry and Yan, 2015). However, a study that used GreenFeed units for Holstein bulls reported a lower correlation of 0.62 (Callegaro et al., 2022). Genetic correlations between GreenFeed recorded CH_4 production and CO_2 production have been estimated at 0.68 ± 0.04 for daily means and 0.65 ± 0.05 for weekly means (van Breukelen et al., 2023). Thus, CO_2 production measurements may provide additional information on CH_4c and CH_4 production and can for example be useful to enlarge CH_4 datasets, by including cows that had only CO_2 production recorded with CO_2 as a predictor trait.

5.4.10 Genetic correlations for methane concentrations between parities

Although the estimated phenotypic correlations between parities were weak to moderate, the genetic correlations were strong and ranged from 0.86 ± 0.20 to 0.97 ± 0.15. The weak phenotypic correlations suggest that environmental effects segregate the measurements between parities and thus by measurements taken far apart in time. Nonetheless, the strong genetic correlations show that cows with a breeding value for low CH_4c in first parity are also likely to have a breeding value for low CH_4c in later parities. The genetic correlations are stronger than what has been reported by Manzanilla-Pech et al. (2021), who reported strong genetic correlations between first and second parity, and second and third parity (0.91 ± 0.11 and 0.85 ± 0.22, resp.), but a moderate correlation of 0.48 ± 0.21 between first and third parity. Furthermore, the genetic correlations between CH_4c and the reported breeding goal traits were similar over parities. High genetic correlations between parities suggest that a repeatability model, including a within and across parity permanent environmental effect, could be sufficient in analysing data across parities, as an alternative to using a multiple-trait model (Strabel et al., 2005), allowing for more simplistic models to be used for new breeding value estimations.

5.4.11 Implications

Genetic correlations are used to predict the consequences of selection, to investigate undesired responses in traits, and to develop informed breeding goal traits for methane. Thereby, the estimates are essential to be able to make informed selection decisions in the future. The results of this study show that the genetic correlations of milk production traits (MY, FY, PY, F%, P%, and ECM), DMI, and BW with sniffer recorded CH_4c are weak. Thus, undesired responses in other traits are expected to be minimal and lowering CH_4c while improving the other breeding goal traits is possible. For example, a genetic correlation between CH_4c and F% of 0.21 means that only 4% of the variation in CH_4c can be explained by differences in F%, whereas for MY with a genetic correlation of -0.04 less than 1% of the variation in CH_4c can be explained by differences in MY. Thereby, the weak genetic correlations also show us that the investigated breeding goal traits are not informative as predictors for CH_4c and endorse the importance of direct recording of CH_4 emissions.

The weak genetic correlations also have implications for trait definition. One trait that has been investigated in the literature is residual CH_4 (Berry et al., 2015; de Haas et al., 2017; Manzanilla-Pech et al., 2021). Residual CH_4 can be phenotypically or genetically adjusted for production, BW, and/ or DMI. By doing so, animals that have a lower CH_4 emission regardless of their level of production, BW, and DMI can be more easily identified. However, weak correlations between CH_4c and the adjustor traits indicate that little variance is explained by the adjustor traits and thus most likely there will be little to no difference in the breeding values for sniffer CH_4c and residual CH_4. Therefore, sniffer CH_4c may be preferred for breeding programs over residual CH_4, because interpretability of sniffer CH_4c is easier.

5.5 Conclusions

This study provided insights into the genetic correlations between enteric CH_4c and DMI, BW, and milk production traits of dairy cows. The results indicate that while the genetic correlations between CH_4c and these traits are generally weak, there are positive correlations, particularly with FY and F%. However, because of the weak relationships, the effect of selection for lower CH_4c on FY and F% is expected to be small. Strong genetic correlations of CH_4c between different parities suggest consistency in breeding values for CH_4c emissions across parities and the genetic correlations with DMI, BW, and milk production traits were similar over parities. Overall, the weak genetic correlations between CH_4c and production traits suggest that it is feasible to select for lower CH_4c, while improving milk production and other economically important traits.

Acknowledgements

This work was funded by the TKI Agri & Food project LWV19155 and the partners CRV and FrieslandCampina, and was supported by the Climate Approach project funded by the Dutch ministry for agriculture, nature and food quality (LNV) (project number BO-53-003). The authors thank the dairy farms for participating in this research, Theo van Hattum, Pieter Hogewerf, and Harm Wemmenhove (Wageningen Livestock Research, Wageningen, the Netherlands) for installing and maintaining the sniffers, and Gerrit Seigers (IT Development, Wageningen, the Netherlands) for setting up the data platform for sniffers. The authors have not stated any conflicts of interest.

6

General discussion

Worldwide, the pressure on reducing agricultural methane (CH_4) emissions is increasing. Most agricultural CH_4 emissions come from enteric emissions of ruminants, which have proven to be difficult and costly to mitigate. However, animal breeding offers a promising cost-effective solution. A limiting factor in implementing the mitigation of CH_4 emissions in breeding programmes has been the lack of CH_4 phenotypes that are readily available. For this thesis, we used sniffers to phenotype dairy cows by measuring CH_4 and carbon dioxide (CO_2) concentrations from breath and belching in the feed bins of automated milking systems (AMS). The collected dataset consisted of 74,569 weekly average CH_4 measurements from 7,139 cows on 68 commercial Dutch dairy farms. With the data we have estimated genetic parameters for various trait definitions and investigated phenotyping strategies. In addition, we estimated genetic correlations with CH_4 production records from the more accurate GreenFeed (GF) CH_4 recording method and with milk production traits, dry matter intake (DMI), and body weight (BW). The results from this thesis are currently being used to develop breeding value estimations for CH_4 in the Netherlands from which a first breeding value will be introduced in 2025.

This general discussion will bring together the results published in the research chapters of this thesis and will reflect on how sniffer CH_4 phenotypes can be implemented in breeding programmes that aim to apply direct selection to reduce enteric CH_4 emissions. The general discussion starts by reflecting on challenges that are associated with large-scale on-farm recording of CH_4 from dairy cows with sniffers. Following this, genetic parameter estimates for various CH_4 phenotypes defined from sniffer measurements are summarised and discussed, and recommendations for phenotyping strategies are given. The discussion concludes with recommendations to ensure a successful implementation of breeding for low CH_4 emissions of dairy cows, targeting adoption by commercial farmers, methods to accelerate sustainable breeding, and methods to quantify the impact of breeding for low CH_4 emissions.

6.1 Phenotyping with sniffers

The research chapters of this thesis are a result of analyses on an extensive dataset of CH_4 concentrations recorded with sniffers that measured CH_4 and CO_2 concentrations from the feed bin of AMS on Dutch dairy farms. Sniffers are used to measure emissions of cattle, however, to date sniffers have mostly been used in research projects on both research and commercial dairy farms (Breider, 2018; Huhtanen et al., 2015; Lassen and Lovendahl, 2013; Lopez-Paredes et al., 2020; Madsen et al., 2010; Pszczola et al., 2017; van Engelen et al., 2018). Different research groups use various type of sniffers, which are sometimes developed by the research group together with companies with expertise on sensors for recording emissions in various types of industries in and outside of agriculture (an overview of available

sniffer devices is given in Jonker et al. (2020)). As a results, large differences exist in hardware, recording set-up, and handling of raw measurements. Understanding these differences is essential to draw conclusions from the data, and to correctly apply the phenotypes in industry breeding programmes in the near future.

6.1.1 Practicalities of using sniffers for methane recording individual cows

The practical use of sniffers is still in its infancy, and the experiment conducted for this thesis on 68 dairy farms highlighted several lessons to be learned for on-farm phenotyping practices with sniffers. In the following paragraphs, common factors that impact data quality will be discussed and recommendations will be given on how to minimise their impact to be able to apply sniffers more effectively in ongoing and future research and industry projects.

6.1.1.1 Challenges of measuring on dairy farms

During the recording of the data used in this thesis, a common issue was the accumulation of dust (e.g., from the pelleted food) and moisture in the sampling tube, which resulted in restricted air passage through the tube and thereby disturbed sampling and measurements. Restriction of the sampling tube generally occurred from twice a week to only once every several weeks depending on the farm. Differences between farms most likely were a result of differences in the barn environment, dimensions of the feed bin, location of the inlet of the sampling tube, and the type of feed that was provided in the AMS.

An easy solution to prevent restriction of the sampling tube is to use pressured air to blow accumulated dirt back into the feed bin, preferably daily. Cleaning the sampling tube with pressured air can be carried out manually, as was done during the data recording for this thesis, or automated using the pressured air that is used to open and close the gates of the AMS (i.e. sneezing, such as is applied by a research group in Denmark (Manzanilla Pech, 2023)). An additional way of avoiding sampling problems from dust is to place a filter where the gas sample reaches the device. In addition, it is recommended to place an extra filter halfway through the sampling tube, that can easily be visually inspected for dirt or moisture and replaced when needed (Jonker et al., 2020). Preventing dirt from reaching the CH_4 and CO_2 gas sensors is important to increase the durability of the gas sensors. To further increase the durability and stability of the gas sensors, the casing of the sniffers should sufficiently protect against moisture and pests such as mice and flies, for example, by adhering to IP65 standards. In addition, a stable temperature should be maintained within the casing and moisture should be actively removed from the sample. A stable temperature should also aim to avoid freezing of the moisture from condensation in the sampling tube in cold environments, which occurred in the sniffers used for this thesis during winter months until a technical modification was made.

6.1.1.2 Calibration of gas sensors

Another common issue with sniffers that can cause inaccuracy is drift of the CH_4 and CO_2 sensors, which is the gradual deviation of the sensor's output from its calibrated state over time. Drift is a common issue in using non-dispersive infrared sensors (Dinh et al., 2016), and has, next to CH_4 sensors, for example been reported in the use of near-infrared (NIR) spectroscopy sensors applied on milk samples from AMS (Diaz-Olivares et al., 2020). However, standardised methods to correct for drift in the data and online calibration maintenance techniques are lacking and should be developed for different applications independently.

The nondispersive infrared (NDIR) sensors used to measure CH_4 in this thesis usually started to drift after three months or more, going up to a year. When drift is notable, usually as skewness of the recorded data over time where the mean moves towards zero or the minimum moves away from zero, the gas sensors will have to be recalibrated using flacons of known CH_4 or CO_2 concentrations. For animal breeding purposes, small amounts of drift are not problematic as for breeding value estimations drift of the sensor will be identical for all cows measured within a farm during the same period of time. Therefore, drift can be corrected for in modelling, by fitting effects between farms and over time. In any case, it is recommended to calibrate NDIR sensors at least once every half year (depending on the type of sensor and manufacturer information), however, from practice this may be required more frequently (e.g. monthly) for some devices. To determine when sniffers need to be re-calibrated the data should be checked regularly.

In addition, the CH_4 and CO_2 sensors in the sniffers used for this thesis did not drift to the same degree, which may result in spurious relationships between the CH_4/CO_2 ratio and its component traits. The CH_4/CO_2 ratio is important as it is often used to estimate CH_4 production from CH_4 concentration measurements (Madsen et al., 2010). Thus, when performing analyses on the ratio, care must be taken in drawing conclusions in the presence of sensor drift. In addition, we observed that a large proportion of CO_2 concentrations were recorded at the upper detection threshold of 10,000 ppm for the sniffers used for this thesis. Thus, most likely, the sensors were not able to record sufficient variation in the high CO_2 emitting cows. For future studies that aim to record CO_2 emissions from breath, it is recommended to use CO_2 sensors with a higher detection threshold, for example, up to at least 25,000 ppm and possibly up to 50,000 ppm (Jonker et al., 2020; Kjeldsen et al., 2024).

6.1.1.3 Head movements

During recording, further inaccuracies in sniffer measurements can come from differences in head movements of the cow, as in many AMS the cow can fully remove its snout from the feed bin to look around while its being milked (Huhtanen et al., 2015; Wu et al., 2018). A

study by Huhtanen et al. (2015), has shown that head movement behaviour is highly repeatable, however, it is unknown if the behaviour is also heritable. If the head movement behaviour would be heritable and when there is a moderate to high genetic correlation with CH_4 concentrations selection for low CH_4 emitting cows may result in changes in cow behaviour in the AMS. To confirm this, it could be of interest to investigate if a trait for head movement is heritable and genetically correlated to CH_4 production. For example, a trait could be defined using recording by GF units as the time that the cows' head is not in the GF unit during a record as a percentage of the total time of the record.

To account for head movement of cows in the AMS, recently an algorithm has been developed to filter out anomalies due to head movement based on CO_2 concentration measurements (Bokde et al., 2023). The algorithm was validated against CO_2 emissions recorded by GF units and successfully detected more than 90% of the irregularities in the CO_2 data. Therefore, the algorithm appears to be an effective method to correct for head movements and could potentially be applied to the data used for this thesis, where CO_2 and CH_4 concentrations were simultaneously measured. However, the algorithm was tested on data from sniffers with higher detection thresholds for CO_2. Therefore, the effectiveness of the algorithm on the data used in this thesis should first be evaluated before application.

Hypothetically, an alternative method to account for head movements of the cows may be through the amount of concentrate that is fed in the AMS. Based on the amount of concentrate fed, it could be determined how long a cow needs on average to finish eating concentrate, after which the likelihood of the cow lifting its head from the feed bin becomes larger. Consequentially, the data after that period of time could be discarded. For the studies reported in this thesis, data on concentrate provided by the AMS was not available and thus this theory should first be further investigated. Another potential approach could be to apply computer vision techniques, using cameras to monitor head movements of the cows in the AMS to correct the sniffer measurements for head movement. Although algorithms for detecting head movement in AMS from images or video have not yet been developed, computer vision techniques show great potential and are increasingly utilised to track cattle for various purposes such as lameness detection (Barney et al., 2023; Kang et al., 2021).

6.1.1.4 Feeding behaviour

Other animal behaviours that may affect the measurements include differences in feeding behaviour. Methane emissions are generally highest during the six hours following feed consumption when the volume of feed being digested in the rumen is greatest (Hristov and Melgar, 2020). However, sniffers take spot measurements and the time since last feeding is unknown. Hypothetically, some cows may prefer to rest after consuming feed and visit the AMS never directly after a feeding event, whereas other cows may always prefer to visit the AMS directly after a feeding event. This would result in the first cow having lower

concentrations recorded by the sniffer, even though its total CH_4 production over a full day might be identical to or higher than that of other cows, which could go undetected by the sniffer. In current large-scale phenotyping with sniffers and GF units, it is not possible to distinguish such differences unless individual DMI is also recorded. When DMI would be recorded, the CH_4 data could potentially be corrected for the exact time of feeding, as an alternative to the correction for the hour of recording CH_4 within farm as was done for all research chapters in this thesis. Nonetheless, as DMI is an expensive trait to record, it is unfeasible to record DMI on all farms where CH_4c is recorded by sniffers.

Hypothetically, an alternative method could be based on the number of eructations in breath, as it has been observed that the number of eructations in breath increased during feeding (Garnsworthy et al., 2012a). Based on peaks in CH_4 emissions, the number of eructations per, for example, five minutes could be determined. This parameter could potentially be of interest to investigate if the last feeding time can be predicted based on the number of peaks in CH_4 emissions per five minutes from a number of repeated breath measurements for each individual cow. However, to the author's knowledge, this approach has not been investigated to date.

As an alternative, future studies could also first attempt to define a trait based on the average time between feeding and CH_4 recording, using data from farms that record both DMI and CH_4, for which genetic correlations with CH_4 emissions could be estimated. If the time between feeding and CH_4 recording would turn out to be heritable and repeatable, negative genetic correlations would indicate that high CH_4 emitting cows generally have less time between feeding and CH_4 recording. This would indicate that the time between feeding and CH_4 recording should be accounted for to avoid changes in animal behaviour when breeding for lower CH_4 as recorded by sniffers. As an alternative, in future studies genetic correlations with emissions recorded by for example respiration chambers (RC) can aid to observe if there is re-ranking in cows, where low correlations could indicate that there is an impact of differences between feeding times and AMS or GF visits. If there is re-ranking of cows, it is important to further develop methods that can correct for differences in the time since last feeding, as otherwise genetic selection may be less effective than expected by not being able to correctly pinpoint low CH_4 emitting cows.

6.1.1.5 Agreement between sniffers and GreenFeed

To determine if sniffer CH_4 phenotypes, despite their inaccuracies, are suitable indicators of total CH_4 production, we estimated the genetic correlation between sniffer-recorded CH_4 concentrations (ppm) and GF-recorded CH_4 production (g/day) in **Chapter 3**. Both methods record spot-samples of CH_4 emissions, however some differences between the methods exist that impact their accuracy. Sniffers generally use a low-capacity pump of 0.6 to 4 L/min to

transport breath samples of the cow to the sensors through the sampling tube that is placed in the feed bin of the AMS. Because of the pump's low capacity, the total breath of the cow is not captured and measured. On the other hand, GF units use a flux method and sample all concentrations from breath around the full head of the cow at 1,560 to 2,250 L/min (Huhtanen et al., 2015). Furthermore, the GF units use an additional sensor that can detect the moments when the cows' head is inside the unit. Therefore, phenotypes by GF units are expected to provide a more reliable spot-sample estimate of the cows' total CH_4 production.

In **Chapter 3**, we estimated a genetic correlation between sniffer-recorded CH_4 concentration (ppm) and GF-recorded CH_4 production (g/day) of 0.76. In addition, the heritabilities for sniffer and GF phenotypes were similar. These results indicated that the sniffer concentration measurements provide a good indicator to reduce CH_4 production in breeding programmes, assuming that the GF phenotype is equal to true total emissions. The strong genetic correlation indicates that selection on low CH_4 concentrations recorded by sniffers will result in a reduced CH_4 production as recorded with GF units. Furthermore, because Greenfeed units record head movements of the cow and thus the GF measurements are less influenced by head movements, it suggests that with sufficient records by sniffers there may be little difference when adjusting or not for head movement.

Overall, due to their low specificity, sniffer sensors currently show the most promise for use in phenotyping for genetic improvement programmes. The inaccuracies in the measurements prevent drawing conclusions from smaller datasets, which are typically collected for experimental nutritional or behavioural studies. Repeated and long-term data collection on individual animals, combined with modelling to correct for environmental effects, is essential to draw meaningful conclusions from the collected data (Bovenhuis et al., 2018; Wu et al., 2018). Furthermore, by comparing the sniffer phenotypes with measurements from GF units, we assumed that GF units measure total CH_4 production. However, this is a strong assumption, as GF units also provide multiple short-term measurements throughout the day and do not capture total emissions (Huhtanen et al., 2015). Previous studies have published estimates of strong phenotypic correlations between measurements by GF and RC (Hristov et al., 2018; Velazco et al., 2016). Nonetheless, to gain more confidence in the relationship between GF phenotypes and total CH_4 production, it would be of interest to also estimate genetic correlations between GF and RC phenotypes, or directly between sniffer and RC phenotypes.

6.1.2 Processing of raw measurements

In our experiment, where we measured CH_4 emissions with sniffers on approximately 70 dairy farms, CH_4 was recorded every five seconds, resulting in more than a million records collected each day. All records were pushed real-time to a cloud database using the mobile network, making the data directly accessible without needing to visit the farm where the

sniffer was installed. However, with long-term recording, the data would also include periods where functionality was hindered due to factors such as dirt in the sampling tube, freezing of the system from moisture accumulation in winter, the sampling tube being pulled out of place by cows, or the need for gas sensor recalibration. When multiple sniffers record long-term on several farms, it becomes unfeasible to monitor, select, and filter data manually.

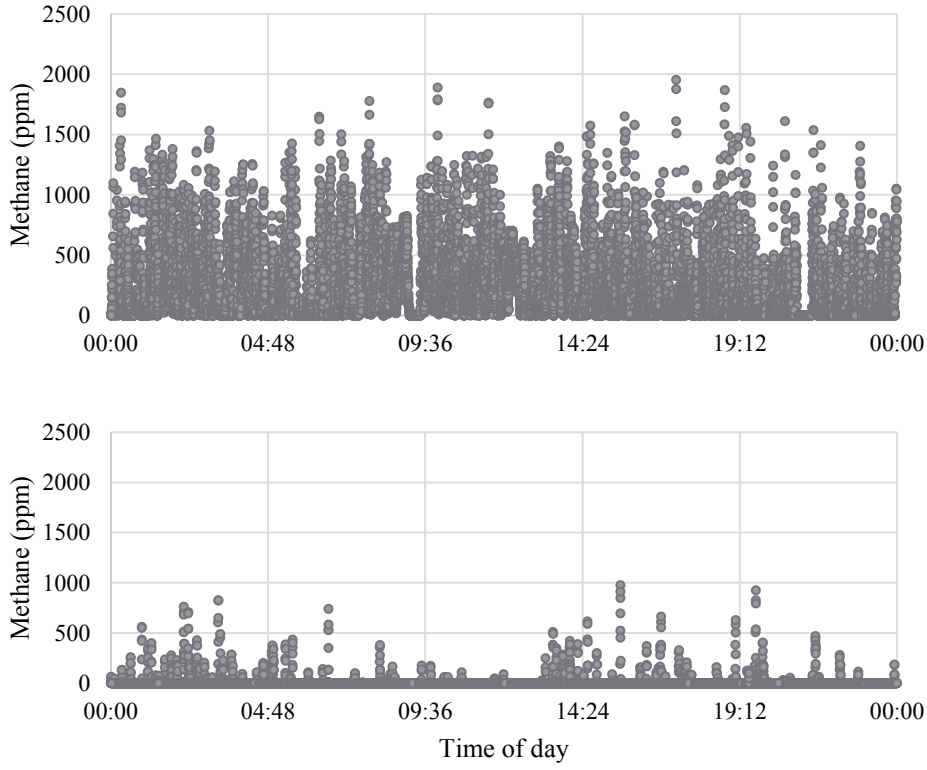

Figure 6.1. Methane concentrations (ppm) measured by sniffers in two milking robots, during two separate periods of one day. On the top, peaks in methane are continuously being measured, whereas in the bottom figure recorded concentrations are low and often nearing zero, which can occur due to blockages in the sampling tube or drift of the methane sensor

During the data recording for this thesis, hindered functionality would result in a decrease of measured concentrations (Figure 6.1), and in the most extreme cases zero concentrations would be measured continuously. As a method of quality control, automatic data filtering steps were applied to discard data during moments where data recording was most likely

hindered (**Chapter 4**). While downloading the data from the cloud database, it was grouped by farm and hour, and discarded if: 1) the mean was below 30 ppm CH_4, 2) the inter quartile range was below 200 ppm CH_4, 3) the maximum was above 3,500 ppm CH_4, or 4) if at least 30% of the data would fall within the range of the first and second mode, plus and minus 10 ppm CH_4. In addition, individual outliers were discarded that fell outside of the upper and lower 0.001st quantile. Together, the criteria ensured that measured CH_4 concentrations would fall within the expected range of cows' CH_4 breath concentrations (Jonker et al., 2020), while maintaining sufficient variation in measurements as would be expected from cows that are visiting the AMS throughout the day. The filtering steps applied in this thesis resulted in the data moving closer to normality, and more of the variance going into the genetic variance, as opposed to the residual variance or permanent environmental variance, in the genetic analyses.

Alternative methods to account for anomalies in sniffer data, due to for example tube blockages or sensor drift, are absent in the literature. Initial research projects using sniffers typically involved short recording periods (one to two weeks) with regular sensor calibration, and therefore accumulation of dust and drift of sensors were less problematic. As discussed before, an algorithm has been described in the literature to filter out anomalies due to cows' head movements (Bokde et al., 2023). However, it is unknown whether this algorithm is robust enough to filter out anomalies caused by blocked sampling tubes, sensor drift, and other interferences. Thus, there is a need for further development and comparison of methods and algorithms to filter and standardise sniffer concentration measurements used for genetic evaluations. These methods should be robust across various types of sniffers, different farms, and different brands and types of AMS. Developing versatile algorithms for data filtering of long-term CH_4 recording in AMS will improve data quality and reduce labour required to check and filter data.

Nonetheless, ideally each issue should be tackled at its source to increase the accuracy of sniffer measurements. For example, blocking of sampling tubes can be overcome by cleaning the tube automatically by using pressured air from the AMS gate to blow through the sampling tube when the gate closes after each milking (Manzanilla Pech, 2023). For drift, real-time automatic alerts can be set up to detect differences in the mean and variance of measured concentrations over time (e.g. between days). When an alert is triggered, the sniffer should be recalibrated. Addressing these issues directly would reduce the number of data gaps due to later data filtering. The data filtering steps used in this thesis often resulted in gaps ranging from an hour to several days or even weeks, until technicians could address the issues on-farm. Such gaps are undesirable because they create inconsistencies in lactation records for some cows, which can affect the reliability of future breeding values for bulls by reducing the number of records for some daughters. However, since not all cows are in the same lactation stage within a farm, the effect on the estimated reliability is expected to be

minimal if the recording period is sufficiently long to capture an adequate number of repeated records per daughter, as recommended in **Chapter 2**. For example, for bulls with 10 recorded daughters, having five weekly records per daughter would be sufficient.

6.1.3 Linking methane measurements to individual cows

Next to the filtering of raw data, processing steps are required to determine the individual cow that a CH_4 record belongs to. Generally, sniffers do not record cow identification numbers and cows are identified based on the corresponding AMS record that was taken at the same moment in time. For the studies reported in this thesis, an automated method similar to the method reported by van Engelen et al. (2018) was applied (for more details see **Chapter 2**). In this method, the AMS visits are aligned to the CH_4 data in such a way that recorded concentrations were the highest during AMS visits and lowest in between AMS visits. However, evaluation of intermediate results by the human eye was required to decide on the most likely alignment, which becomes infeasible for increasingly large datasets.

In the literature, several other methods have been described that are used to combine the two separate data sources. For example, a study by Difford et al. (2016) described an algorithm that can account for fixed or variable time shifts between timestamps from the clock of the sniffer and the clock of the AMS. The algorithm detects sudden and large changes in the recorded gas concentrations to determine cow entry times and aligns this with moments that the AMS was occupied by a cow. Variable time shifts, next to clock shifts, are useful to account for lags in concentrations being picked up by the gas sensors. In the experiment reported in this thesis, we observed lags of up to one minute, which could become longer or shorter depending on the length of the sampling tube. To account for lag, the first minute of milking was always discarded in this thesis to avoid measuring merely background concentrations. However, correcting for variable time shifts for each sniffer could provide a useful alternative and would prevent the unnecessary discarding of data when lags are shorter than one minute.

A limitation of the algorithm by Difford et al. (2016) is that the algorithm was not tested in applications with long-term recording, where tube blockages and drift of sensors may occur. Furthermore, the algorithm is not well-suited for large-scale continuous recording, because also this algorithm requires evaluation of intermediate results by the human eye to make subjective clock-alignment decisions. More recently, a study by Milkevych et al. (2022) described a more robust and fully automated algorithm applied to data from multiple commercial dairy farms in Denmark, using a matched filter approach. The described algorithm also accounts for noise, such as head movements during milking. The method shows great potential to be tested and applied on the dataset described in this thesis and could assist researchers and industry working with similar datasets. Furthermore, by standardising

raw sniffer data processing methods over different datasets, in addition to standardising phenotypes, inference of the results across different experiments would become easier.

In this thesis, we relied on information from the AMS to acquire cow ID. However, cow identification through the AMS may not always be readily available. Determining cow ID without AMS information can be addressed using a standalone RFID reader. However, this is often discouraged as it may interfere with the AMS RFID reader. As an alternative, computer vision techniques could be applied to read the ID of individual animals from their ear tags during milking in the AMS (Bastiaansen et al., 2022). The advantage of using cameras is that the recordings could potentially be used simultaneously to detect head movements, as discussed above. Nonetheless, computer vision techniques should be used with caution, as the currently developed algorithms are more likely to result in false identification compared to the AMS RFID reader. For example, in the study by Bastiaansen et al. (2022), the precision was 65% and the sensitivity was 41%, but when ear tag numbers were reported, they were correct 93% of the time. Further development of these algorithms is required to improve their accuracy and sensitivity, but the method shows potential to enlarge CH_4 datasets in practices where cow ID cannot be recorded by the AMS or an RFID reader.

6.1.4 Extending the use of sniffers to record cows

Most commonly, sniffers are used to measure CH_4 and CO_2 concentrations in the feed bin of AMS. However, sniffers have previously also been implemented in automated concentrate feeders (Negussie et al., 2017) or head boxes in milking parlours (Calderon-Chagoya et al., 2019) to be able to measure emissions on farms without AMS. In principle, the devices could be used in many applications, however, to phenotype individual animals accurately it is important that the animal remains in a position with its muzzle close to the air inlet of the sampling tube for several minutes to obtain a sufficiently reliable phenotype. In addition, since sniffers have a passive pump, care should be taken to minimise environmental disturbances such as air velocity. To do so, the area around the cow's muzzle can be protected with materials that break the wind and air drafts.

Sniffers and other breath sensors could potentially measure additional gases, which can be easily integrated into currently developed sniffer devices. Expanding the applications of sniffers could help to distribute the costs of the sensors across different targets, making CH_4 recording by sniffers more accessible. Furthermore, a broader applicability could enhance collaborations between genetic research and other projects. For example, studies by Elliot-Martin et al. (1997) and van Erp-van der Kooij et al. (2023) described using breath measurements as a potential health indicator for detecting ketosis in dairy cows. In addition, breath measurements have been recognized as a method to detect paratuberculosis in cattle (Ellis et al., 2014; Weber et al., 2021). Other gases that could be recorded are for example

oxygen (O_2) and hydrogen (H_2). Oxygen is applicable in estimating the heat production of cows (Brouwer, 1965). Heat production reflects a loss of energy, which could otherwise go to milk production, reproduction, or maintenance, and is thus a trait of economic importance. Hydrogen and CO_2 are the principal substrates of CH_4 production in the rumen (Janssen, 2010). Higher H_2 concentrations for low CH_4 emitting cows are usually not found (whilst not feeding CH_4 inhibitors), as other bacteria-archaea networks appear to become active which utilise alternative H_2 pathways (Stepanchenko et al., 2023). In addition, H_2 is more challenging to measure than CH_4, and is heavily affected by sampling scheme, especially under restricted feeding (van Lingen et al., 2023). Thus, H_2 emissions are not directly suitable as an indicator for genetic selection for low CH_4 emissions. However, the H_2 measurements together with measurements on volatile fatty acids can be used to investigate rumen fermentation dynamics and the metabolic physiology of rumen microbes (Islam et al., 2024; Olijhoek et al., 2016; van Lingen et al., 2021). This is also of great interest for investigating and monitoring changes in rumen dynamics as a consequence of genetic selection for low CH_4 emissions.

Finally, moderate to high phenotypic and genetic correlations are often found between CH_4 and CO_2 measured by sniffers (as reported in **Chapter 3** and **Chapter 5**). As a result, when only breath measurements on CO_2 are available, they could potentially be used as an indicator for CH_4 emissions to enlarge datasets. In addition, CO_2 has been suggested as an indicator for DMI or residual feed intake (RFI), as CO_2 measurements are cheaper than direct DMI measurements (Difford et al., 2020; Huhtanen et al., 2021). However, the genetic correlations between sniffer CO_2 and DMI reported in **Chapter 5** were low (0.19 ± 0.23 to 0.29 ± 0.16, over parities, and therefore applying sniffer CO_2 concentration measurements as an indicator for DMI for breeding is likely not effective. Nonetheless, genetic correlations may be higher using more accurate phenotyping methods, such as GF units, and should therefore be re-evaluated for phenotypes from other CO_2 recording methods.

6.1.5 Conclusions

This chapter evaluated the practical use of sniffers for recording CH_4 emissions from dairy cows, highlighting challenges and solutions for on-farm phenotyping. Sniffers face issues such as dust and moisture accumulation and sensor drift, necessitating periodic maintenance and data control to ensure data reliability. Head movements and feeding behaviours of cows can further affect measurements, suggesting the need for algorithms to filter anomalies and potentially using alternative data to investigate their effect on the recorded CH_4 concentrations. Despite the possible inaccuracies, the genetic correlation between CH_4 concentrations measured by sniffers and CH_4 production measured by GF units was strong, indicating the suitability of sniffers for phenotyping for genetic selection. Nonetheless, effective data processing and automatic filtering is essential for managing the large volumes

of records that are collected whilst ensuring data quality. Additionally, expanding sniffer capabilities to measure other gases has the potential to provide further insights into cow health and efficiency. Overall, for successful implementation in breeding programmes, optimising sensor maintenance and data processing are crucial to improve the accuracy of measurements.

6.2 Using Sniffer Phenotypes in Breeding Programmes

In **Chapter 2** of this thesis, we have defined various traits derived from data recorded by sniffers. These traits included mean emissions, median emissions, log transformed mean emissions, and the CH_4/CO_2 ratio. Additionally, research projects and industry have considered other traits such as peak traits, CH_4 efficiency, and residual CH_4. Determining which CH_4 trait or combination of traits is most suitable for implementation in breeding programmes remains to be answered.

6.2.1. Trait definitions and heritability

The heritability of a trait is an important parameter to judge the effectiveness of genetic selection and higher heritabilities will result in reaching higher reliabilities of breeding values from recorded phenotypes on the same number of animals. Estimates for the heritability of CH_4 related traits published in the research chapters of this thesis and in the literature show large heterogeneity and ranged from 0 to 0.45 (**Table 1.1**). To be able to compare estimates, it is essential to consider differences between phenotyping methods and trait definitions. Generally, one or more of these factors will differ between studies, resulting in differences in parameter estimates.

For example, in the chapters of this thesis we have described estimates on averages per AMS visit (**Chapter 2**), per day (**Chapter 3**), and per week (**Chapter 2, 3, 4,** and **5**). In general, averages over longer recording periods yield a higher heritability and repeatability, as can be observed in **Chapter 2** where the heritability for visit mean CH_4c was 0.13 ± 0.01, and for weekly mean CH_4c on the same dataset was 0.32 ± 0.01. However, while longer averages yield higher heritabilities, the reliability of breeding values remains similar whether the records are averaged or analysed as repeated measurements, as was demonstrated in **Chapter 2**. In addition, in **Chapter 3** we reported a genetic correlation of 1.00 ± <0.01 between mean CH_4 per day and per week (coming from the same data). Thus, albeit the heritabilities are lower for daily averages, the cows are expected to be ranked similar for visit mean CH_4c and weekly mean CH_4c when modelled using repeatability models.

Other differences in genetic parameter estimates can result from model differences, such as whether records within and across lactations are used. When including several lactations in the genetic analyses, ideally the repeatability model should include a within lactation permanent environmental effect and an across lactation permanent environmental effect. In

our analysis, we observed that including only a within-lactation permanent environmental effect inflated the heritability estimates (0.34 ± 0.02 versus 0.18 ± 0.03, **Chapter 3**). If the dataset is not large enough to separate within-lactation and between-lactation permanent environmental effects, the heritability can be estimated by splitting the data by lactations, using a multi-trait model that separates first, second, and remaining lactations.

Overall, using only the magnitude of the heritability to judge trait applicability for breeding programmes is not appropriate. Each trait definition and model should be compared carefully, to judge the expected progress from estimating breeding values or from including that trait into a breeding programme. Several other aspects unrelated to the magnitude of the heritability are of importance, which are: the ease of measurement, the ease of interpretation for industry and farmers, and genetic correlations with other important breeding goal traits. The following paragraphs will discuss advantages and disadvantages for each trait.

6.2.1.1 Mean emissions

The most reported traits for CH_4 emissions measured by sniffers are mean CH_4 concentration and mean CH_4 production. Several previous studies have estimated the heritability for mean CH_4 emissions, where heritability estimates for CH_4c ranged from 0.11 ± 0.02 to 0.26 ± 0.11, and heritability estimates for CH_4 production ranged from 0.12 ± 0.03 to 0.24 ± 0.15 (Table 1.1). Also for mean CH_4 emissions, differences in trait definitions reported in the literature are common, for example: 1) the study by van Engelen et al. (2018) analysed average CH_4c per AMS visit, 2) the studies by Pszczola et al. (2017) and Sypniewski et al. (2021) averaged emissions per day, 3) the studies by Breider et al. (2019), Difford et al. (2020), Lassen and Lovendahl (2016), Manzanilla-Pech et al. (2022b), and Zetouni et al. (2018) analysed averages per week, and 4) the study by Lassen et al. (2016) analysed a single weekly average.

Overall, heritability estimates for sniffer mean CH_4c and sniffer mean CH_4 production are moderate, and while both traits are easy to record, CH_4c is easier to explain to industry and farmers. Sniffers are not able to directly measure CH_4 production and therefore it is generally approximated using CO_2 concentrations as a tracer gas through a formula that assumes a constant efficiency of energy utilization for different metabolic functions (Madsen et al., 2010). However, this assumption is not always met and can result in CH_4 production to be overestimated, on average by 17% for efficient compared with inefficient cows, favouring the inefficient cows (Huhtanen et al., 2020). The formula also incorporates information on BW and energy corrected milk yield (ECM). Thereby, the sniffer CH_4 production phenotype is also likely to reflect differences in BW and ECM, possibly resulting in stronger genetic correlations with the associated traits that are not a result of differences in true CH_4 production. The relationships with associated traits should most likely be corrected for before

including the trait in breeding programmes, further complicating the interpretability of the trait.

6.2.1.2 The ratio of methane to carbon dioxide

One of the underlying traits in estimating sniffer CH_4 production is the ratio between CH_4 and CO_2 (Madsen et al., 2010). The ratio is of interest because energetically it would be optimal to metabolise all feed carbon to the production of milk, body weight gain, pregnancy, and CO_2 and the carbon extracted as CH_4 reflects the energy that is lost. Therefore, the CH_4/CO_2 ratio describes the proportion of excreted carbon that is not metabolised as CO_2 and a lower ratio reflects a more efficient cow. However, phenotypic and genetic correlations published in **Chapter 2** between mean CH_4c and the CH_4/CO_2 ratio were negative (-0.09 ± 0.01 and -0.56 ± 0.22, respectively). Additionally, the heritability and repeatability for weekly mean CH_4/CO_2 were low (0.02 ± 0.01 and 0.15 ± 0.01, respectively). These findings suggest that the CH_4/CO_2 trait is not a suitable trait to use for CH_4 mitigation through animal breeding. However, the experimental set-up used for data collection for this thesis may have been unsuitable to reliably estimate the CH_4/CO_2 ratio (see the discussion of **Chapter 2**). For example, a study by Lassen and Lovendahl (2016) published a higher estimate of the heritability for CH_4/CO_2 of 0.16 ± 0.04 and a genetic correlation with sniffer CH_4 production of 0.83 ± 0.14. This implies that with an improved experimental setup the CH_4/CO_2 ratio might provide an accurate indication of energy losses in dairy cows in other experiments. Due to the limitations in the data used, the ratio trait was not considered as a phenotype and not used to estimate CH_4 production by the equation of Madsen et al. (2010) for this thesis.

6.2.1.3 Peaks in methane emissions

Other traits of interest for breeding, commonly reported in the literature, are traits based on peaks in CH_4 emissions. Cows are known to belch or eruct gases from the rumen at intervals of around one minute (Garnsworthy et al., 2012a). Only considering peaks in CH_4, as opposed to an average of the full AMS visit, ensures that cows' belches are analysed with possibly less disturbances from background CH_4 concentrations in between belches. However, questions remain about how peaks from belches reflect total CH_4 production. Only analysing the peaks in CH_4 emissions might miss the emissions that are present in breath. The relationship between mean CH_4 emissions and CH_4 peaks has not been extensively analysed and remains unclear.

Peak traits were initially not analysed on the data used for this thesis, because of the recording interval up to 35 seconds for the sniffers used for **Chapter 1** and **Chapter 2**, which makes it likely that the maxima of peaks are missed when measuring the emissions. Nonetheless, as an exercise in understanding the data and the relationship between peaks and mean CH_4c better, some peak traits were later defined and analysed (Meijer et al., 2021). The analysed peak traits were: 1) the maximum CH_4 concentration recorded during milking (max CH_4), 2)

the mean of all records that were within the 25% of highest concentrations recorded (25% highest CH_4), and 3) the mean of peaks in CH_4 concentrations, where peaks were defined as two increasing measurements followed by two decreasing measurements using the *findpeaks* function from the package *pracma* (Borchers, 2019) in R 4.0.2 (CH_4 peaks). All traits were calculated from AMS visits and thereafter averaged per week, including only weeks with a minimum of five records per trait. The traits were analysed using bivariate models (identical to the model used in **Chapter 2**), including 13,099 records (on each trait) from 1,424 individual cows.

From these analyses, we observed slightly higher heritabilities for the maximum and peak traits, compared to the mean CH_4 trait, although within the range of the SE (Table 6.1, Meijer et al. (2021)). The repeatability of mean CH_4c was 0.57 ± 0.01 and the trait 25% highest had the highest repeatability of 0.61 ± 0.01. Max CH_4 and CH_4 peaks had lower repeatabilities of 0.55 ± 0.01 and 0.54 ± 0.01, respectively. A lower repeatability for the max CH_4 was expected, because this trait included only one record per visit, whereas the other traits were based on an average of multiple records during a visit. Therefore, the max CH_4 trait is expected to be more heavily affected by environmental noise in the data. Nonetheless, this did not result in a lower heritability.

Table 6.1. Parameter estimates for six different methane (CH_4) traits[1] based on weekly means. Including heritability (h^2) with standard errors (se) on the diagonal, phenotypic correlations above the diagonal and genetic correlations below the diagonal (adapted from Meijer et al. (2021))

	Mean CH_4c	Max CH_4	25% highest	CH_4 peaks
Mean CH_4c	0.33 ± 0.02	0.88	0.96	0.91
Max CH_4	0.96	0.37 ± 0.02	0.97	0.89
25% highest	0.99	0.99	0.36 ± 0.03	0.93
CH_4 peaks	0.97	1.00	1.00	0.33 ± 0.02

[1]Mean CH_4c: mean CH_4 concentrations; Max CH_4: mean of maximum CH_4 concentrations per visit; 25% highest: mean CH_4 of the 25% of highest concentrations recorded; CH_4 peaks: mean of CH_4 peaks

In addition, phenotypic and genetic correlations between the peak traits and mean CH_4c were high (≥ 0.88 for phenotypic and ≥ 0.96 for genetic correlations, Meijer et al. (2021)). Thus, although the 35 second recording interval of the sniffers used for **Chapter 2** of the thesis may introduce noise in the peak traits, the results suggest that the mean trait and the peak traits can be used interchangeably in breeding programmes as animal will be ranked similarly and the traits have similar heritabilities. Furthermore, based on the high phenotypic and genetic correlations with mean CH_4 emissions (Table 6.1), it appears that cows that produce

the highest peaks in concentrations also on average have the highest total concentrations measured.

Traits based on peaks in emissions are also common in the literature. However, the definition of a peak can differ between studies. For example, peak traits can be calculated from: 1) the area under the curve, 2) a maximum value, or 3) an average of multiple peaks. Therefore, with new definitions of peak traits it is always important to first investigate the relationship with total CH_4 production, to ensure that genetic selection has the desired outcomes. The heritability for peak traits has been estimated in the literature at 0.11 ± 0.03 when derived from CH_4c and at 0.12 ± 0.04 when derived from CH_4 production (Lopez-Paredes et al., 2020) and at 0.12 ± 0.01 when derived from CH_4c (Saborío-Montero et al., 2019). This is lower than the heritability estimates for peak traits reported in Table 6.1. A possible explanation could be that the study of Saborío-Montero et al. (2019) used a single average of CH_4 and the study by Lopez-Paredes et al. (2020) used weekly averages in a repeatability model, but from short recording periods of 14 to 21 days per farm. Thus, also here the phenotyping strategy, trait definition, and choice of model may have resulted in heterogeneity in heritability estimates, making the heritability by itself an unsuitable parameter to support decision making in which trait to include in breeding programmes. All in all, both mean CH_4c and peak traits are easy to interpret, their heritability and repeatability estimates were similar, and the genetic correlations between the traits were high, indicating that both would be suitable to apply in breeding programmes and could possibly be used interchangeably after standardisation.

6.2.1.4 Methane efficiency and residual methane

Other traits that have been mentioned in the literature are CH_4 efficiency traits. Efficiency traits are usually defined as CH_4 as a ratio to production or intake, making them independent of the denominator trait. Well known efficiency traits for dairy cows are CH_4 intensity (g CH_4/ kg milk) and CH_4 yield (g CH_4/ kg DMI), as reviewed by de Haas et al. (2017). However, for breeding programmes, using ratio traits is generally discouraged as a multi-trait approach is likely to result in higher genetic gain than by selecting for ratio traits and responses in ratio trait can be hard to interpret as it is not immediately apparent if the response is due to a change in the nominator or denominator trait (Veerkamp, 2002; Veerkamp et al., 2013; Zetouni et al., 2017).

An alternative to using ratio traits is using a residual CH_4 trait (Berry et al., 2015; de Haas et al., 2017; Manzanilla-Pech et al., 2021). Residual CH_4 can be phenotypically or genetically adjusted for production, BW, and/ or DMI. By doing so, animals that have a lower CH_4 emission regardless of their level of production, BW, and DMI can be more easily identified. Similar approaches are often applied on DMI data, to adjust DMI for production and other energy sinks, resulting in RFI (Koch et al., 1963; Pryce et al., 2014; Veerkamp et al., 1995).

In these approaches, phenotypic RFI is generally modelled as DMI minus predicted DMI based on, for example, milk production and growth (Tempelman et al., 2015; Veerkamp and Emmans, 1995). Genetic RFI is generally modelled by performing a genotypic regression of DMI on milk production and other energy sinks (Kennedy et al., 1993). Similar methods have also been used to make live weight independent of body condition score (Veerkamp and Brotherstone, 1997) and to adjust longevity for milk production (van der Linde et al., 2007).

A study by Manzanilla-Pech et al. (2021), showed that genetic correlations between residual CH_4 (adjusted for metabolic BW and ECM) were not only zero with BW and ECM, but also with DMI. Thus, selecting for residual CH_4 adjusted for metabolic BW and ECM would also not have an impact on DMI. Furthermore, in the study by Manzanilla-Pech et al. (2021) residual CH_4 was positively correlated with RFI, indicating that lower CH_4 emitting animals were more efficient at converting feed. Heritability estimates of residual CH_4 were similar to estimates of mean CH_4 concentrations and production and were 0.11 ± 0.03 and 0.23 ± 0.06 for residual CH_4 concentrations and 0.21 ± 0.03 and 0.16 ± 0.04 for residual CH_4 production (Manzanilla-Pech et al., 2022b; Manzanilla-Pech et al., 2022c), and the phenotypes had moderate to high genetic correlations with CH_4c and CH_4 production (0.69 ± 0.12 to 0.82 ± 0.07, Manzanilla-Pech et al. (2022c)). Thereby, residual CH_4 showed potential for inclusion in breeding programmes.

To investigate the applicability of residual CH_4 concentrations (RMC) using the data described in this thesis, I performed genetic regressions on CH_4c. Following the method of Kennedy et al. (1993) and Veerkamp and Brotherstone (1997) the genetic regression coefficients of ECM, BW, and DMI (b_{ECM}, b_{BW}, b_{DMI}) were calculated using the (co)variances estimated with bivariate models in **Chapter 5** for first parity cows. Thereafter, genetic covariances and genetic correlations were estimated between RMC and the ten breeding goal traits reported in **Chapter 5**.

By applying genetic regressions on CH_4c, the heritability of RMC was estimated at 0.21, which is similar to the heritability for CH_4c estimated in this thesis (0.18, **Chapter 3**) and to heritabilities from the literature reported above. The estimated genetic correlations between RMC and the ten breeding goal traits were similar for RMC adjusted for DMI, ECM, and BW, for ECM and BW, or only for ECM (Table 6.2). As expected, after adjusting for ECM, BW, and DMI the RMC trait had no genetic correlations with its regressors. Nonetheless, there was still an undesirable correlation with milk fat percentage of 0.22 and with milk protein percentage of 0.10. Thus, although fat and protein yield are accounted for in the calculation of ECM, it appears that this is not sufficient to avoid a weak selection response in fat or protein percentage when selecting for RMC. In addition, although RMC was corrected for ECM, the RMC trait did not become independent of the underlying traits of

ECM, namely milk yield (MY), protein yield (PY), and fat yield (FY) and genetic correlations with MY, PY, and FY were slightly stronger with RMC than CH_4c (although all correlations were weak).

Therefore, CH_4c is likely a more suitable trait for implementation in breeding programmes than RMC because interpretability is easier, as it is hard to explain that selection responses in MY, PY, and FY may still occur when selecting for improved RMC adjusted for ECM. Furthermore, by reporting a trait that reflects reductions in total CH_4 production, genetic progress for the breeding goal trait can be more easily aligned with government targets that aim to reduce the total CH_4 production from agriculture. By expressing traits only as a residual or as an efficiency trait, there is a risk that total enteric CH_4 emissions continue to increase when the number of cows does not decrease in response to individual cows becoming more efficient.

Table 6.2. Genetic correlations between CH_4 concentration (from **Chapter 5**) or residual CH_4 concentration (RMC), and other breeding goal traits on first parity cows[1]. RMC was genetically standardised for DMI, ECM, and BW, ECM and BW, or only ECM

	CH_4c	RMC (ECM/ BW/ DMI)	RMC (ECM/ BW)	RMC (ECM)
CH_4c	1	1.00	1.00	1.00
CO_2c	0.81	0.72	0.73	0.73
DMI	0.06	0	0.04	0.04
MY	-0.04	-0.08	-0.08	-0.08
ECM	0.04	0	0	0
PY	<0.01	-0.04	-0.04	-0.04
FY	0.12	0.08	0.08	0.08
P%	0.10	0.10	0.11	0.11
F%	0.21	0.22	0.22	0.22
BW	-0.04	0	0	-0.04

[1]CH_4c = methane concentration; CO_2c = carbon dioxide concentration; DMI = dry matter intake; MY = milk yield; ECM = energy corrected milk yield; PY = protein yield; FY = fat yield; P% = protein percentage; F% = fat percentage; BW = body weight; RMC = residual methane concentration

In addition, the genetic correlations between RMC and CH_4c were 1.00 after rounding to two decimal places. This indicates that no variance in CH_4 was explained by ECM, BW and DMI, which can be expected from the very weak estimated genetic correlations between CH_4c and ECM, BW, and DMI. Therefore, there will likely be little difference in the breeding values for CH_4c and RMC and for genetic progress there will be no difference in picking one or the other breeding value for implementation. Residual traits are of more interest for traits with

moderate to strong genetic correlations with the traits by which they are adjusted. Therefore, for CH_4 traits from other CH_4 recording methods, the applicability of RMC should be re-evaluated. For the sniffer CH_4c trait used in this thesis, there appears to be no added value in using a RMC trait.

6.2.2 The effect of different phenotyping strategies on genetic progress

Another important aspect that has an impact on the genetic progress is determined by the moment in a cows' lactation when CH_4 is recorded and for how many days or weeks. To get a better understanding of when to record CH_4 emissions, phenotypic and genetic lactation curves can be informative. In **Chapter 2, 3, and 4** phenotypic lactation curves were plotted for CH_4 emissions recorded by sniffers and by GF units, where we observed a steep increase in emissions during the first days in milk (DIM), whereafter the emissions stabilised or slowly decreased. Thus, absolute values in CH_4 emissions change during the lactations, but also the underlying relationships between genetic and phenotypic variances, which is reflected in the heritability, have been shown to vary during the lactation (Manzanilla-Pech et al., 2022a; Pszczola et al., 2017; Sypniewski et al., 2021).

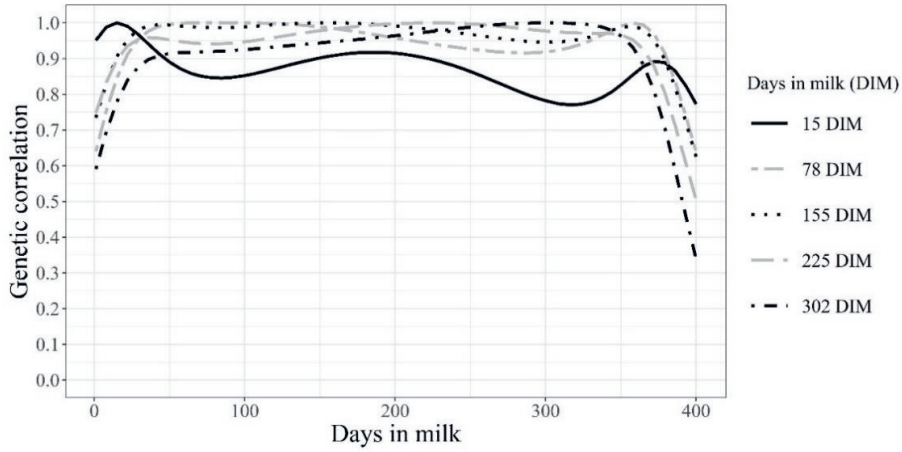

Figure 6.2. The genetic correlations between CH_4 concentration recorded on 15, 78, 155, 225, or 302 days in milk (DIM) and recorded on all other DIM

In **Chapter 4** of this thesis, we modelled genetic lactation curves and investigated the effect on different recording and modelling strategies. In that chapter, we showed that all genetic correlations between DIM were positive and most genetic correlations were high, except for some correlations at the beginning and end of the lactation. This can also be observed in

Figure 6.2, which was created using the genetic correlations that were estimated in **Chapter 4**. In this figure, it can be observed that mid lactation records (between 15 and 302 DIM) are highly correlated with all other DIM. Only records taken before 15 DIM and after 350 DIM, may have lower correlations with other lactation stages. Thus, very early and very late lactation CH_4c might not reflect mid lactation CH_4c and ideally emissions should be recorded during mid-lactation, or modelled with random regression models if some cows only have emissions recorded in early or late lactation. However, the extremes in the beginning and end of the lactation may partly result from an artefact in using Legendre polynomials in a random regression model and should be further investigated as has been extensively discussed in **Chapter 4**. In addition, the genetic correlations for CH_4c are the highest between measurements taken closely together in time (Figure 6.3). On average, the genetic correlations are above 0.95 for measurements taken less than 125 days apart in a lactation. For longer periods of time between measurement days, the genetic correlations will continue to decrease steadily. Thus, estimated breeding values (EBV) will be the most accurate around the period of time for which CH_4c records are available.

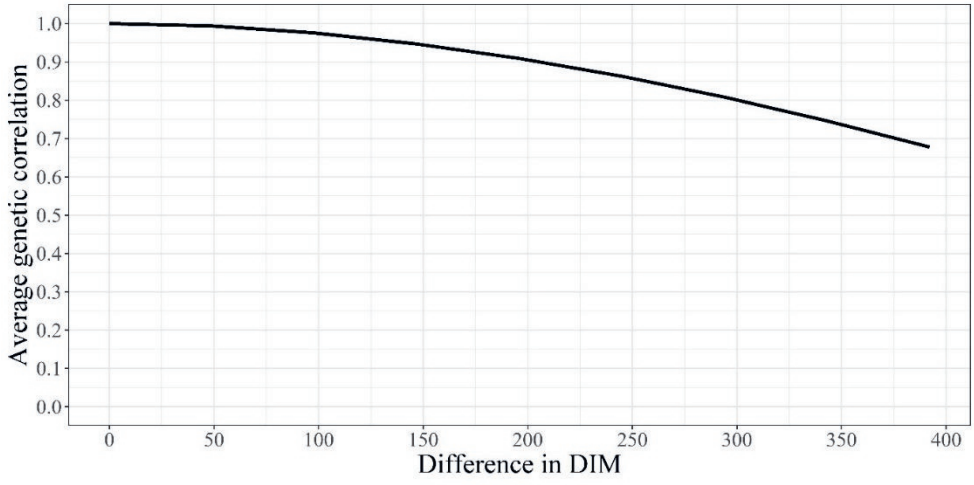

Figure 6.3. Average genetic correlation of CH_4 concentrations when the recordings are 1, 8, 15, …, 400 days in milk (DIM) apart in time

The decay in genetic correlations can influence the reliability of breeding values estimated from different models (**Chapter 4**). To be specific, the reliability is likely to be overestimated by fixed repeatability models when cows would be recorded only in early lactation (up to ± 50 DIM). This is because the fixed repeatability model assumes genetic correlations of one between all DIM. Therefore, when some cows are recorded during early lactations and others

during other stages of lactation, a random regression model should be preferred to avoid lower than expected genetic gains and an overestimation of the reliability of the breeding values for some cows. In addition, when it is practically possible to apply short recording periods for all cows in a similar lactation stage, for example in seasonal calving systems, it is preferred to record mid-lactation as this would yield the highest reliabilities.

Table 6.3. The predicted reliabilities for cows with own performance information and sires with phenotyped daughters. The reliabilities for cows are derived from the number of weekly records, and the heritability and repeatability for weekly means. The reliabilities for sires are derived from the cows' reliability from n records, and the number of daughters

Number of records per cow	Predicted reliability cow	Number of daughters per sire	Predicted reliability sire
1	0.18	5	0.19
		10	0.32
		15	0.41
		100	0.82
5	0.31	5	0.29
		10	0.46
		15	0.56
		100	0.89
10	0.34	5	0.32
		10	0.48
		15	0.58
		100	0.90
25	0.36	5	0.33
		10	0.50
		15	0.60
		100	0.91
50	0.37	5	0.34
		10	0.50
		15	0.60
		100	0.91

To determine the minimum duration of recording, in **Chapter 2** we estimated that to reach the Dutch breeding value publication threshold of 50% for sires at least 25 visits or five weeks from ten daughters per sire should be recorded. However, here we used the model including only a within parity permanent environmental effect, whereas in **Chapter 4** we showed that the heritability estimated using a within and a between parity permanent environmental effect was lower. To observe the effect of the difference in parameter estimates on breeding values, in Table 6.3, I provide the calculated reliabilities from the updated heritability and

repeatability estimates (0.18 and 0.48, resp.). In this table, it can be observed that by recording five weeks from ten daughters a reliability of 0.46 will be achieved. To reach a 50% reliability, 12 daughters per sire would have to be recorded with each five weeks of recording. Thus, although the heritability is lower, the impact on the number of recorded daughters required to reach a sufficiently high reliability for a sire is small and the estimates are similar.

6.2.3 Genetic correlations with other breeding goal traits

Genetic correlations are important to understand how selecting for lower CH_4 emissions might affect other breeding goal traits and are thereby essential to make informed breeding decisions. In **Chapter 5** we estimated genetic correlations between CH_4 and important milk production traits, DMI, and BW. Next to the traits published in **Chapter 5**, there are several other important traits in the breeding goal that are related to health, fertility, conformation, production, and feed efficiency. In the Netherlands, these traits are combined in the Dutch national index NVI (CRV (2023).

To investigate the relationships between CH_4c and all breeding goal traits included in the NVI, additional genetic correlations were estimated using the multiple trait across country evaluation (MACE) procedure. The MACE procedure is used to evaluate bulls for one trait across countries by Interbull (Interbull Centre, 2017), but can also be used to estimate genetic correlations between deregressed sire EBV of different traits (Larroque and Ducrocq, 1999; Schaeffer, 1994). Genetic correlations estimated by the MACE procedure were used, because they allow for estimating correlations based on the EBV without the need for the data and models for all traits in the NVI. For the NVI traits, deregressed sire EBV from CRV were used as input and for the CH_4 traits EBV were estimated based on a univariate analysis similar to what has been described in **Chapter 2**. The MACE correlations were estimated using a pedigree relationship matrix for all sires that had at least one daughter with a CH_4 recording. However, as the analyses included many sires with few daughters, the estimates are likely associated with a large uncertainty and should be interpreted with caution (SE were not provided, as they are not estimated by MACE). For all reported traits based on the EBV including CH_4, a higher value is desirable. This is contrary to the estimates for CH_4c published in **Chapter 5**, where a lower value for CH_4 was desirable.

Table 6.4. Genetic correlations based on the multiple trait across country evaluation (MACE) procedure, between Dutch national index (NVI) traits and weekly mean methane concentrations combining information from all parities using a weighted index for CH_4 concentrations (CH_4c). A higher value is desirable for each trait, and thus for methane a higher value reflects lower emissions

	CH_4c
NVI	0.08
Milk yield (kg)	-0.11
Fat yield (kg)	-0.08
Protein yield (kg)	-0.04
Lactose yield (kg)	-0.18
INET*	-0.07
Longevity (days)	-0.01
Functional longevity (days)	-0.01
Udder (pnt)	-0.09
Feet & legs (pnt)	-0.06
Direct calving ease (pnt)	0.19
Maternal calving ease (pnt)	0.07
Interval calving-first insemination (pnt)	0.23
Interval first-last insemination (pnt)	0.21
Conception rate (pnt)	0.14
Conception rate heifers (pnt)	0.30
Direct vitality (pnt)	0.23
Maternal vitality (pnt)	0.06
Udder health (pnt)	0.06
Claw health (pnt)	0.23
Survival (pnt)	-0.22
Saved feed cost for maintenance (euro)	0.21

* Dutch production index

Similar to the genetic correlations with milk production traits estimated in **Chapter 5**, the MACE correlations between CH_4 and MY, FY, PY, and the Dutch production index (INET) were weak (Table 6.4). The correlation with FY showed an undesired negative trend in the MACE correlations. This is similar to the positive correlations reported in **Chapter 5** (0.12 ± 0.08 to 0.23 ± 0.10), because for the CH_4 EBV used in the MACE procedure a higher EBV reflected lower CH_4c. Interestingly, the MACE correlations for lactose yield were higher than for FY and PY and, therefore, it might be of interest to estimate correlations between CH_4

and lactose on real data to derive more accurate estimates. In the literature, one estimate of a genetic correlation between sniffer CH_4c and lactose yield has previously been reported of 0.32 ± 0.06 (van Engelen, 2018), indicating that indeed an undesirable genetic correlation between CH_4c and lactose yield may exist. Nonetheless, in the same study the genetic correlation with lactose percentage was weak (0.06 ± 0.06) and the MACE correlations with lactose yield estimated here were also weak. Thus, the relationship with lactose is expected to be weak and selecting for lower CH_4c is not expected to have a significant impact on lactose yield.

Similar to the MACE correlations between CH_4 and milk production traits, the MACE correlations between CH_4 and all other NVI traits were weak and ranged from -0.22 for survival to 0.30 for conception rate heifers. Estimates of genetic correlations in the literature between CH_4 emissions recorded by sniffers and health, conformation, and fertility traits are scarce. A genetic correlation between sniffer-recorded CH_4 and survival (i.e. calf vitality) has to date not been published and should be further investigated using real data as the direction of the MACE correlation was undesired and indicated lower survival when breeding for reduced CH_4 emissions. Nonetheless, the relationship between the two traits was weak, and at most 12% of differences in CH_4c may be attributed to differences in survival. The other fertility and health traits provided desired, although weak, MACE correlations with CH_4, and thus appeared to not form a bottleneck when selecting for CH_4 mitigation.

The MACE correlation between sniffer-recorded CH_4 and saved feed cost for maintenance was positive, indicating that cows with lower CH_4 concentration are more feed efficient. Previous studies have also shown favourable correlations between CH_4 production and RFI, ranging from 0.26 ± 0.08 to 0.76 ± 0.09. However, a study by Difford et al. (2020) reported positive genetic correlations between CH_4c and RFI in Danish herds (0.42 ± 0.23 to 0.69 ± 0.15), whereas the genetic correlations were negative for phenotypic and genetic RFI (-0.69 ± 0.38 and -0.55 ± 0.41, resp.) but not for single step RFI in Dutch herds (0.46 ± 0.36). The analyses by Difford et al. (2020) on the Dutch cows comprised a subset of DMI recorded cows that were included in these analyses, thus the relationships with RFI should be further investigated to confirm the relationship between RFI and CH_4 emissions of Dutch dairy cows.

Even though the MACE correlations were weak, the correlations do pinpoint some relationships that should be further investigated using real data, to come to estimates with smaller uncertainty. Especially the relationship between CH_4 and survival is of interest, as this relationship is undesired, whereas relationships with the other health traits and fertility traits were all desired. All in all, selecting for lower CH_4 emissions is not expected to have a large effect on other NVI traits, and thus at the same time genetic improvement in all NVI traits remains possible while simultaneously selecting for lower CH_4 emissions.

6.2.5 Conclusions

Various phenotypes have been defined from raw sniffer data, including mean CH_4 emissions, the CH_4/CO_2 ratio, peak traits, CH_4 efficiency, and residual CH_4. Heritability estimates of these traits reported in the literature vary significantly, however, this is most likely largely a result of differences in phenotyping strategy, resulting trait definitions, and modelling. Analysing the traits on an equal number of records using the same raw data resulted in similar heritability estimates and high genetic correlations between all traits, except the CH_4/CO_2 ratio. This suggests that after standardisation most traits can be used interchangeably in breeding programmes. Overall, most traits show potential for implementation in breeding programmes, however some considerations should be made relating to the interpretability of the trait. For example, CH_4 production derived from sniffer CH_4c measurements may be biased by BW and ECM, which should be corrected for. In addition, residual CH_4 adjusted for ECM still revealed undesired correlations with fat and protein percentages, complicating its use. Therefore, these traits require further validation before implementation, and careful consideration should be made of their possible indirect effects on other breeding goal traits. Directly using the mean CH_4c as a phenotype guarantees easy interpretability. Genetic correlations between mean CH_4c and other breeding goal traits, estimated using the data and the MACE procedure, were weak. The weak correlations suggested that selecting for mean CH_4c recorded by sniffers can be implemented in national breeding programmes to reduce CH_4 emissions while maintaining overall breeding goals.

6.3 Implementation of Breeding for Low Methane

In the previous chapter, we have seen how the genetic parameters estimated in this thesis can be helpful in setting up breeding strategies to mitigate CH_4 emissions using sniffer phenotypes. However, several questions remain that are essential to ensure that estimated breeding values will be adopted in practice to mitigate CH_4 emissions.

6.3.1 Incentives for methane reduction

Usually, traits that are included in breeding goals have an economic value, as maximizing farm profit is defined as the key breeding goal (Cole et al., 2021). The economic value, thereby, helps to determine the relative importance of each trait which is applied as a weighting factor in selection indices. It is likely that carbon taxes will become enforced for all industries, including agriculture, which could make CH_4 mitigation economically beneficial. Although carbon taxes are common throughout Europe (Figure 6.4), they have not yet been implemented for the agricultural sector. However, this is likely to change in the near future, with Denmark being the first European country to announce the introduction of a carbon tax for agriculture from 2030 (Olsen, 2024). In the meantime, societal and governmental pressure to reduce greenhouse gas emissions in agricultural practices, may

nudge the agricultural community towards a decision to include CH_4 emissions in a breeding goal with non-market value through desired gains before carbon taxes become enforced (Boichard and Brochard, 2012; Brascamp, 1984).

Other incentives to mitigate enteric CH_4 emissions can come from the industry. For example, milk processing companies could give higher prices for milk with a lower carbon footprint, which includes a farms' herd having a lower-than-average CH_4 emission. This strategy is likely to be implemented in the Netherlands, where farmers use a tool called the 'Kringloopwijzer' to keep track of on-farm emissions (including CH_4, CO_2, nitrogen, and phosphate) and farms with low emissions are rewarded through milk prices (de Haan and Verloop, 2021). Currently, in the Kringloopwijzer tools are available to estimate CH_4 emissions of a farm based on the number of cows, and management information (e.g. information on feed ration). However, by including breeding values in the Kringloopwijzer, differences between herds based on information about individual animals could be included. Similarly to the Kringloopwijzer, a Danish based company that collects milk from Denmark, Sweden, the United Kingdom, and Germany introduced a 0.04 eurocent milk payment increase related to greenhouse gas emissions in 2023 (Arla, 2023). If this would be linked to breeding values, it can be a strong incentive for farmers to select for low CH_4 emitting cows.

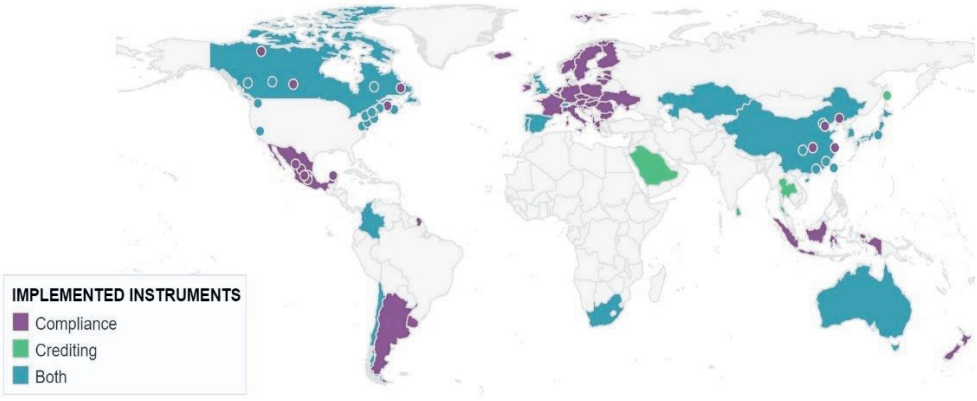

Figure 6.4. Carbon pricing instruments around the world in 2024. Source: https://carbonpricingdashboard.worldbank.org/

6.3.2 Accelerating sustainable breeding

As the initial effects of climate change are already apparent, there is a great urgency in reducing carbon emissions to prevent further worsening (IPCC, 2021). Genetic selection has the advantage that its effect is cumulative and permanent, however, changing populations

through genetic selection can be a slow process, with noticeable improvements becoming visible only over several generations. Nonetheless, several opportunities exist to increase and accelerate genetic progress.

6.3.2.1 Indirect selection for a lower carbon footprint of dairy

Once the economic value or desired gain of CH_4 has been determined, CH_4 can be implemented in new or existing indexes. For example, a new index can be developed that targets environmental sustainability. In such an index, next to reducing direct CH_4 emissions, other NVI traits can be optimised so that the carbon footprint is minimised. For instance, the Irish Beef Cattle Federation (IBCF) created an Economic Breeding Index (EBI) using current breeding goal traits, which indirectly reduces greenhouse gas emissions without using direct CH_4 measurements. This index achieved an economic gain of 20 euros net profit per cow per year while leading to a 2% reduction in carbon footprint (ICBF, 2018). Similarly, in the Netherlands, it was shown that breeding for increased production in livestock has indirectly reduced the environmental impact by 1% a year per kg of product produced (Mollenhorst and De Haas, 2019). This was also observed in studies in the United Kingdom, Ireland and Canada, that showed that the emission intensity will be reduced by 1% per year through their national breeding programmes (Amer et al., 2018; Bell et al., 2014a; Richardson et al., 2021a). A study in New Zealand on eight breeding goal traits indicated that increased production efficiency through selecting for farm profitability indirectly helps to mitigate CH_4 emissions by -0.04 kg CO_2-equivalents/ kg milk protein equivalent/cow/year (Zhang et al., 2019). Thus, developing an environmental sustainability index shows potential to support continued genetic progress in production, fertility, conformation, and health traits, while also indirectly targeting a reduction in CH_4 emissions.

Nonetheless, by passive genetic gains for CH_4 based on current breeding strategies, as described by the studies above, mitigations targets as set by the Dutch government will not be met. However, combining indirect selection for a lower carbon footprint with direct selection on enteric CH_4 emission traits can provide a powerful tool to accelerate progress in reducing greenhouse gas emissions. When estimating the carbon footprint of other breeding goal traits, it is important to also consider greenhouse gas emissions from other processes, such as feed production and manure management, through a life cycle assessment. Not accounting for these emissions may otherwise lead to bias in the estimated emissions (van Middelaar et al., 2014). To address trade-offs, breeding indices aimed at optimizing the reduction of greenhouse gas emissions from economically important traits should combine bio-economic models with life cycle assessments, as demonstrated by Shi et al. (2024).

6.3.2.2 Applications in other countries and possibilities for collaboration

High concentrations of CH_4 in the atmosphere are a global issue and do not only impact the area where the CH_4 was produced. As animal production in Europe, North America, and Oceania intensified significantly over the 19th and 20th century, countries on these continents should make substantial efforts to reduce their emissions to reach the climate goals set by the Paris Agreement (Clark et al., 2020; Lynch and Garnett, 2021). Additionally, due to continued population growth in countries in Asia, Africa, and Latin America it is expected that the number of cattle in these regions will continue to rise in the coming decades and that production will be intensified (Robinson et al., 2011). Therefore, new initiatives to reduce emissions should be implemented globally and should also include animal production systems in the global south. Currently such initiatives are being developed by, for example, the Global Genetics Program together with ICAR, where CH_4 phenotypes and genotypes that are collected world-wide will be gathered in a shared database to use for genetic evaluations within and across countries (WUR, 2024). Shared databases with many records across countries can help countries with scarce recording of CH_4 to setup breeding for low CH_4 and can also help countries with CH_4 recording to accelerate breeding for CH_4 mitigation by improving the accuracy of estimated breeding values.

However, applying genetic selection to reduce CH_4 emissions across countries world-wide comes with unique challenges (Manzanilla-Pech et al., 2021; Richardson et al., 2022). Dairy production systems are generally very diverse over different climates and many different dairy breeds are kept next to the widespread Holsteins. To overcome these challenges, a joint dataset with data from various countries is likely required to be much larger than within country datasets to be able to obtain sufficiently high reliabilities of breeding values across countries (de Haas et al., 2017; Richardson et al., 2022). Due to the urgency in reducing enteric CH_4 emissions, phenotyping projects are commencing world-wide and collating this data is expected to result in a sufficiently large dataset with CH_4 records on ten thousands of cows. In addition, combining data across countries has previously been done successfully for other traits, for example to increase the reliability of dry matter intake in countries with scare or no recording (Berry et al., 2014; de Haas et al., 2015).

In combining data across countries, there are several ways to deal with phenotypes that are recorded by different systems and reported in different units. Most commonly, multi-trait models are applied, where the phenotypes are analysed as separate traits in an international evaluation as is also done by the MACE procedure. Multi-trait models can simultaneously deal with genotype by environment interaction, which may be induced by large variation in management systems, feed, climates, and/or recording techniques between countries. Regardless to the use of these models, it is essential to have a number of phenotypes recorded in each country, breed and production system that breeding values are estimated for. Alternatively, similar CH_4 traits can be standardised per country to analyse the data as a single

trait. A study by Manzanilla-Pech et al. (2021) has shown that CH_4 production was highly correlated to genetically standardised CH_4, which was standardised using within and across country genetic standard deviation. However, the dataset was not sufficiently large enough to estimate genetic correlations between CH_4 production across countries and within country. These genetic correlations should first be estimated to judge if there are genotype by environment interactions between countries or if implementing a single across country CH_4 trait in international genetic evaluations is possible in specific cases.

6.3.2.3 Using genomic selection

Ideally, genetic progress within and across countries is accelerated by applying genomic prediction (Manzanilla-Pech et al., 2021). Genomic prediction has been developed in the last two decades and adds information on the genomics of individual animals to common mixed models, usually derived from single nucleotide polymorphisms (SNP). In genomic prediction, a reference population is used consisting of cows which have phenotypes and are genotyped, which is applied as a training dataset. From the training dataset, SNP effects are estimated, which are consequentially used in a prediction equation for cows or bulls without phenotypes but with genotype information (the selection candidates). Thereby, also for bulls without phenotyped daughters breeding values can be estimated as long as they are genotyped. Nowadays, genomic selection is widely applied in dairy cattle breeding, including in genetic evaluations across countries (Interbull Centre, 2022; Palucci et al., 2023). The accuracy of genomic predictions can be improved by increasing the number of recorded animals in the reference population, by recording animals within families that are relevant for selection (e.g. from sires with many daughters), by increasing marker density, and by increasing the heritability by reducing environmental effects (de Haas et al., 2012). Likewise, it has been shown that sharing data on CH_4 phenotypes and genotypes across countries can be a powerful method to increase the reliability of genomic prediction within and across participating countries (de Haas et al., 2015; Lund et al., 2011). Thereby, genomic prediction can also help to accelerate the genetic progress for CH_4 mitigation in the Netherlands, through increasing the reliability of breeding values for bulls without or with a low number of daughters with CH_4 phenotypes. Routine genotyping of all CH_4 recorded cows on farm will be essential to be able to implement genomic prediction for CH_4.

6.3.3 Quantifying the expected effect of genetic selection to mitigate methane

For this thesis, we phenotyped individual dairy cows using sniffers. Sniffers measure CH_4 and CO_2 concentrations in the air in ppm and are not able to directly provide an estimate in g/day. Knowing the actual reduction that is achieved by breeding (in total g/day) is important, for example, when farmers need to quantify their emission reductions for the industry or governments. Quantifying emissions will also help to set guidelines in the desired reductions and is needed to form incentives to reduce emissions.

6.3.3.1 Formulas to convert ppm to g/day

Models have been developed that enable the conversion from concentration measurements by sniffers in ppm to an estimation of the emissions in g/day. For example, Chagunda et al. (2009) have described a formula based on an average tidal respiratory volume, whereas Madsen et al. (2010), Suzuki et al. (2021), and Kjeldsen et al. (2024) have described formulas based on CO_2 as a tracer gas in combination with the CH_4/CO_2 ratio. Which formula is applicable depends on the method of recording and other data that is available and should be assessed for each individual application. Nonetheless, using formulas to estimate emissions in g/day from sniffer measurements in ppm may have some disadvantages. For example, Huhtanen et al. (2020) showed that the formula by Madsen et al. (2010) is likely to favour inefficient cows over efficient cows in ranking them from low to high emitting. Most likely, this is a result of biased estimates of CO_2 production from concentration measurements due to differences between cows in their efficiency of energy utilization for maintenance and milk production.

6.3.3.2 Using genetic correlations between predictors and the breeding goal trait

Other strategies that could be investigated to convert the sniffer phenotype to CH_4 emissions in g/day is by looking at relative differences in the breeding value estimates for two different traits. For example, when cows have been recorded by sniffers and GF units, these can be used as a benchmark for estimating the true emissions (assuming emissions measured by GF units are the true emissions). This way the relative scales of the breeding values can be translated into expected responses in g/day for both traits, using the genetic correlation between sniffer-recorded CH_4 concentrations and GF-recorded CH_4 production that was estimated in **Chapter 3**.

Because the number of cows with CH_4 phenotypes are generally low, it may be advantageous to use several CH_4 traits as indicator traits for a general CH_4 breeding goal trait to maximise the reliability of future estimated breeding values. For example, if a few hundred cows are recorded with the more accurate GF units, then the GF CH_4 production (g/day) phenotype can serve as the breeding goal trait. Phenotypes from a large number of cows from other CH_4 traits could then be included as predictor traits for the breeding goal trait using information on the phenotypic and genetic correlations between the breeding goal trait and the predictor traits. In this way, not only sniffer CH_4c phenotypes are valuable as a predictor trait, but also other potential phenotypes based on, for example, MIR (Denninger et al., 2019; Shadpour et al., 2022; Vanlierde et al., 2015), rumen microbial composition (González-Recio et al., 2023; Wallace et al., 2015; Zhang et al., 2020), rumination time, feed intake time (Ramirez-Agudelo et al., 2022), or direct measurements from other sensors (e.g. SF_6, portable accumulation chambers, etc.).

Combining information from multiple sources is recommended, as it not only increases the accuracy of the EBV, but it also results in a better estimation of the true CH_4 emissions when you get closer to the true breeding value (Negussie et al., 2017). Thus, using this method will likely result in a better ranking of cows for their true CH_4 emissions and this is advantageous to quantify emissions for farm and national emission inventories. Further research is necessary to establish the most effective method for converting breeding values, derived from sniffer CH_4 concentration measurements and additional predictors, into actual reductions in total emissions (measured in grams per day) through genetic selection.

6.3.3.3 Estimating the expected impact of breeding for lower methane

An estimation of the expected genetic progress that can be achieved by selecting for lower CH_4 emissions, using CH_4 recording by sniffers, was published in a paper by de Haas et al. (2021). Strong assumptions were made in the selection index calculations about the genetic correlations between sniffer-recorded CH_4 and other breeding goal traits, as at that time the genetic correlations had not yet been estimated due to a lack of data. Therefore, I performed selection index calculations using the spreadsheet with desired gains of van der Werf (2020) used by de Haas et al. (2021) with updated genetic correlations for CH_4c, using the genetic correlations for MY, FY, and PY published in **Chapter 5** and genetic correlations estimated by the MACE procedure for the remaining breeding goal traits. All other parameters were kept equal to the parameters used in the selection index calculations by de Haas et al. (2021). The focus was on scenario 6d published in the study by de Haas et al. (2021), where they modelled selection on maximal CH_4 reduction using information on 100 daughters. The heritability and phenotypic standard deviation (SD) were kept constant, as they were similar to the estimate published for first parity cows in **Chapter 5**.

Through the selection index calculations, the impact of genetic selection for CH_4 intensity (CH_4/ kg milk) was estimated to be larger at a 42% reduction by 2050 compared to 2018 levels, as opposed to the previously published 29% (de Haas et al., 2021). Thus, larger reductions in CH_4 intensity can be achieved while continuously improving other breeding goal traits, because the newly estimated genetic correlations with the other breeding goal traits were weaker than what was previously assumed. However, the trait we want to improve in practice is CH_4 production, for which measurements on sniffer CH_4c can be used as an indicator through the genetic correlation between the two traits of 0.76 **(Chapter 3).** Therefore, an additional scenario was modelled including a trait for GF-recorded CH_4 production. When including a trait for CH_4 production based on sniffer CH_4c measurements as an indicator, the impact of genetic selection was estimated to be slightly lower at a reduction of 38% in CH_4 intensity in 2050.

To model the scenario with GF-recorded CH_4 production, desired gains were used for the GF trait and not for the sniffer trait, whereas all recording was on the sniffer trait without any recording for the GF trait. The genetic correlations between the GF trait and the breeding goal traits were assumed to be the same as the sniffer trait and the heritability and phenotypic SD were kept equal for the two traits, as the estimates for the sniffer and GF published in **Chapter 3** were similar. Sensitivity analyses showed that the estimates were not sensitive to lower and higher phenotypic SD (30 to 125) or moderate differences in correlations between the GF trait and other breeding goal traits. In addition, CH_4 is a scarcely recorded trait and recording on 100 daughters per sire will initially not be realistic. Using desired gains for CH_4 production in combination with genomic prediction can be a practical alternative. The study by de Haas et al. (2021) showed that when using genomic prediction, the genetic improvement in CH_4 intensity was only slightly lower (21% for genomic prediction vs 24% for 100 recorded daughters per bull).

The scenarios above describe the genetic progress in CH_4 intensity (CH_4/ kg milk). In all scenarios, as a constant, the pre-determined desired gain of the maximum possible gain was used for the CH_4 trait and the index weights were included for the current breeding goal traits. As a result, the realised reduction in sniffer CH_4c or GF CH_4 production was equal in all scenarios at 29%. Larger reductions in CH_4 emissions are possible when more selective weight is put on CH_4, such as when assigning economic values of zero to all other breeding goal traits and putting all weight on CH_4 (Figure 6.5). Although putting all weight on CH_4 resulted in a larger reduction in CH_4, it also resulted in a reduced milk production of 4% between 2018 and 2050. Thus, selection merely for lower CH_4 comes at a cost in genetic improvement in the other breeding goal traits, as was also pointed out in the paper by de Haas et al. (2021) and in a study by Gonzalez-Recio et al. (2020). Nonetheless, as the genetic correlations between CH_4c and other breeding goal traits are very weak and lower than what was previously assumed, it is likely that a larger desired gain on lower CH_4 can be used than what was used in the initial scenario's by de Haas et al. (2021).The optimal balance between traits should be determined before implementing the sniffer CH_4 trait in practical breeding programmes.

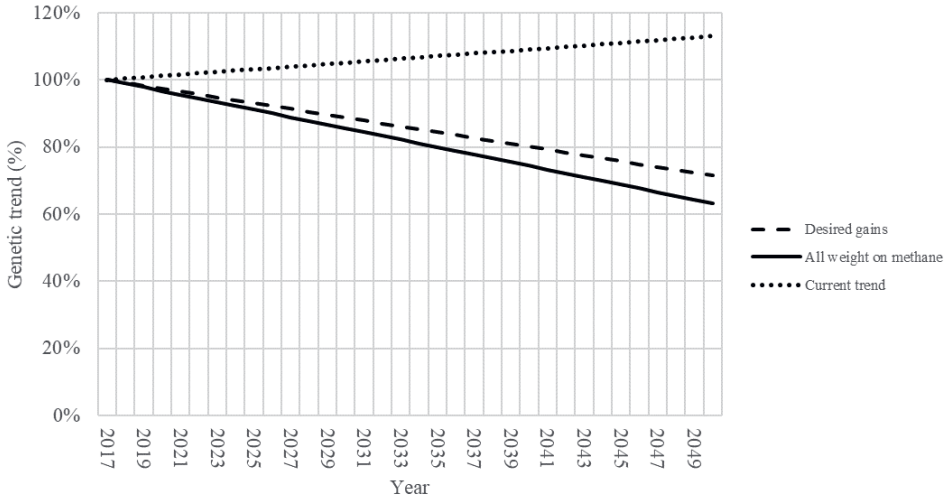

Figure 6.5. The simulated genetic trend in methane (CH_4) production (g/day) for three scenarios: the current breeding goal without active selection for CH_4, using a desired gain of -12.75 for CH_4 (dashed), all selection index weight on CH_4 (solid)

6.3.3.4 The expected impact of breeding on national emission reduction targets

Using the expected responses to selecting for lower CH_4c, a rough estimation can be made of the impact of breeding for lower CH_4 emissions in the Netherlands. From the selection index calculations above, it can be expected that using the scenario above selecting for lower CH_4 emissions would realise a reduction in CH_4 production of 1,239 g/year per cow. An average dairy farm in the Netherlands has 114 cows (CBS, 2024a), which means the reduction for an average herd would be 141 kg/year per herd. On a national level, if genetic selection would be applied for all 1,57 million dairy cows in the Netherlands (excluding youngstock) CH_4 emissions could reduce by a total of 1.95 million kg/year. This is equal to 53 million kg CO_2-equivalents/year, assuming a global warming potential of 27.2 for CH_4 (IPCC, 2021).

The climate goals set by the Dutch government aim to reduce CH_4 emissions by 55% by 2030, as compared to 1990 levels (EZK, 2022). Recently, agricultural emissions were estimated at 24,5 Mton (CBS, 2024b). To reach the 2030 climate goals, agricultural emissions need to be reduced to a level of 17.9 Mton CO_2-equivalents in 2030. This requires a reduction of 1.32 Mton CO_2-equivalents/year over the next five years. If genetic selection would be applied to all dairy cows in the Netherlands, using the scenario described above, a yearly reduction in CH_4 emissions of 53 million kg CO_2-equivalents/year can be achieved, which is

equal to 0.05 Mton CO_2-equivalents/year. Thereby, animal breeding is expected to be able to contribute 4% to the total required reductions for the total agricultural sector to reach the climate targets in the next five years. However, the results depend strongly on the level of implementation by the industry and Dutch dairy farmers and could therefore easily be much lower in practice. This highlights that to reach short-term climate targets set by the government, other strategies should also be explored to reduce enteric CH_4 emissions, for example, targeting CH_4 inhibiting feed additives. Looking at longer periods of time, however, animal breeding is a strong method to permanently reduce enteric CH_4 emissions as the reduction is permanent and cumulative. To be able to reach the long-term targets it is essential that we implement breeding strategies as soon as possible. Furthermore, to increase the impact of genetic selection, additional selection index calculations should be performed to determine optimal selection indices that would lead to a maximum in CH_4 reduction, while still improving other breeding goal traits. Ideally combined with life cycle assessments and bio-economic models to optimise the full index based on the carbon footprint of all traits.

6.3.4 Conclusions

For successful implementation of breeding for low CH_4 emissions of dairy cows, commercial farmers must adopt breeding values, which can be incentivised through economic measures like industry-driven rewards. For instance, milk processing companies can offer higher prices for lower carbon footprint milk to motivate farmers. Furthermore, methods should be investigated that aim to accelerating sustainable breeding. For example, by incorporating selection on direct CH_4 emissions and indirect selection through minimising the carbon footprint of all breeding goal traits. In addition, sharing data across countries has the potential to enhance the reliability of breeding values within and across countries and should be implemented in combination with genomic selection to improve prediction accuracy, thereby accelerating genetic progress. To quantify the impact of breeding for low CH_4 emissions, converting sniffer concentration measurements to emissions in grams per day is crucial. This can be achieved using existing formulas or by using genetic correlations between different measurement methods. Overall, animal breeding can help to significantly reduce enteric CH_4 emissions of dairy cows, contributing to long-term climate goals. However, immediate implementation of these breeding strategies is essential to contribute to both short-term and long-term emission reduction targets.

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Summary

Various strategies have been proposed to reduce enteric methane (CH_4) emissions from ruminants, focusing on areas such as management, feeding strategies, feed additives, vaccination, and animal breeding. Among these, animal breeding currently demonstrates the greatest long-term potential, attributed to its minimal costs of implementation, along with its lasting and cumulative impact. However, incorporating CH_4 into breeding programmes is still at an early stage. An important limitation to practical application has been the lack of phenotyping of CH_4 emissions on enough individual cows to be able to estimate sufficiently reliable genetic parameters, which are required for informed breeding decisions. However, recent innovations have accelerated the collection of CH_4 phenotypes.

For this thesis, enteric CH_4 emissions were measured by 'sniffers' that sample air from the feed bin of milking robots. The latest dataset included 74,569 weekly mean CH_4 concentration (ppm) records on 7,139 cows from 68 commercial dairy farms. As the sniffers only measure concentrations and not the total grams of CH_4 emitted by breath, an additional dataset was analysed that included measurements from GreenFeed (GF) units on CH_4 production (g/day) on 797 cows from 16 farms (four overlapping with sniffers). The general objectives of this thesis were to: 1) define a CH_4 trait from raw sniffer CH_4 concentration measurements, and estimate heritabilities and repeatabilities, 2) investigate the relationship between two CH_4 recording methods (sniffers and GF units), 3) investigate the effect of different recording schemes on the reliability of breeding value estimations, and 4) investigate the relationships between CH_4 and other breeding goal traits.

In **Chapter 2**, genetic parameters were estimated for various traits defined from the sniffer concentration measurements and the number of measurements that would be required to get a sufficiently high reliability of breeding values was estimated. High genetic correlations were estimated between several traits defined from sniffer CH_4 measurements ($\geq$0.78), apart from the genetic correlations with the CH_4/CO_2 trait, which were negative. For weekly mean CH_4 concentrations (CH_4c), we estimated that five records on CH_4c, measured on ten different daughters would be sufficient to achieve a minimum reliability of 50% for the estimated breeding value of a bull.

In **Chapter 3**, genetic correlations between CH_4c recorded by sniffers and CH_4 production recorded by the more accurate but more expensive GF units were estimated. The final data comprised 24,284 GF daily means from 822 cows, 170,826 sniffer daily means from 1,800 cows, and 1,786 daily means from 75 cows by both GF and sniffer (in the same period). Heritability estimates for GF and sniffer were similar, and the genetic correlation between CH_4 recorded by the two recording methods was high and was 0.71 ± 0.13 for daily means and 0.76 ± 0.15 for weekly means. The results indicate that selection based on sniffer data could effectively reduce CH_4 emissions in g/day as quantified by GF. This supports the

potential of using cost-effective sniffer phenotypes in breeding programmes aimed at lowering CH_4 emissions from dairy cattle.

In **Chapter 4**, a comparison was made between genetic parameter estimates for CH_4 emission from a fixed regression repeatability model and a random regression (RR) model. The RR model allowed for varying genetic variances and covariances over a lactation. The results showed that the heritability was highest mid lactation (on average 0.17 ± 0.04), and genetic correlations between lactation stages were high (0.34 ± 0.36 to 0.91 ± 0.08). Permanent environmental correlations deviated greatly over a lactation and ranged between -0.73 ± 0.08 and 1.00 ± <0.01, which highlights that it is most appropriate to model CH_4c with a RR model including a random permanent environmental effect. With many full-lactation daughter CH_4 records for each bull, the reliability was similar for the fixed regression and RR models. However, when data were only available for shorter recording periods at the beginning and end of lactation, using the fixed regression model led to up to a 28% reduction in reliability for bulls. Assuming the fixed regression model when the true (co)variance structure is reflected by the RR model, more than twice as long recording from the start of lactation was required to achieve maximum reliability for a bull. Therefore, applying an overly simplistic model could lead to insufficient recording and lower than predicted genetic gains based on the estimated reliability.

In **Chapter 5**, genetic correlations between enteric CH_4c and dry matter intake, body weight, and milk production traits were estimated. The results indicated that while the genetic correlations between CH_4c and the other traits were generally weak, there were some positive correlations, particularly with fat production traits (fat yield and fat percentage). However, because of the weak relationships, the effects of selecting for lower CH_4c on fat yield and fat percentage are expected to be small. In addition, strong genetic correlations of CH_4c between different parities suggested consistency in breeding values for CH_4c across parities, and the genetic correlations of CH_4c with dry matter intake, body weight, and milk production traits were similar over parities. Overall, the weak genetic correlations between CH_4c and production traits suggest that it is feasible to select for lower CH_4c, while improving milk production and other economically important traits.

In **Chapter 6**, the general discussion, the results of this thesis are put into a broader context, starting at evaluating the practical use of sniffers for recording CH_4 emissions and ending with a reflection on how breeding for low CH_4 emissions of dairy cows can be implemented in practical breeding programmes. The first part of the discussion highlights several challenges in on-farm recording with sniffers, for which suggestions for periodic maintenance and data processing are given to ensure data reliability and to improve the accuracy of measurements. The second part of the discussion describes various phenotypes from raw sniffer data, including: mean CH_4 emissions, the CH_4/CO_2 ratio, peak traits, CH_4

efficiency, and residual CH_4. Here, I discuss that most traits show potential for implementation in breeding programmes, however some considerations should be made relating to the interpretability of each trait and some traits require further validation before implementation. In addition, I describe genetic correlations between mean CH_4c and all breeding goal traits in the Dutch national index. The last part of the discussion focuses on strategies to ensure successful implementation of breeding for low CH_4 emissions of dairy cows. I discuss methods to ensure that commercial farmers adopt breeding values and methods that aim to accelerate sustainable breeding. In addition, I discuss methods to quantify the impact of breeding for low CH_4 emissions, converting sniffer concentration measurements to emissions in grams per day. Overall, animal breeding can help to significantly reduce enteric CH_4 emissions of dairy cows, contributing to long-term climate goals. However, immediate implementation and optimalisation of breeding strategies is essential to be able to contribute to both short-term and long-term emission reduction targets.

Appendices

About the Author

List of Publications

Training and Supervision Plan

Acknowledgements

About the Author

Anouk van Breukelen was born on the 10th of September of 1996, in Bodegraven, the Netherlands. She did a bachelor in Animal Husbandry at the HAS University of Applied Sciences in 's-Hertogenbosch, the Netherlands from 2013 to 2017. In the same year, she started her master in Animal Sciences at Wageningen University, for which she received her degree in 2019. During her masters, she specialised in Animal Breeding and Genetics, with a minor thesis entitled "Genetic analysis of early fertility in Rode Geus cattle", and a major thesis entitled "Genetic diversity and inbreeding in Dutch cattle breeds based on gene bank collections".

After graduating in 2019, she started working as a researcher at Wageningen Livestock Research, working on topics related to cattle breeding for circular dairy farming, detecting lameness with IR thermography using computer vision, and methane emissions of dairy cattle. In 2020, she started her PhD project to further dive into the topic of reducing methane emission through animal breeding in dairy cattle. The results of her PhD are presented in this thesis. Since July 2024, Anouk is continuing her work as a researcher at Wageningen Livestock Research.

List of Publications

Peer reviewed publications

van Breukelen, A. E., Veerkamp, R. F., de Haas, Y., & Aldridge, M. N. (2024). Genetic parameter estimates for methane emission during lactation from breath and potential inaccuracies in reliabilities assuming a repeatability versus random regression model. Journal of Dairy Science. https://doi.org/10.3168/jds.2024-24285

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van Breukelen A. E., Doekes H. P., Windig J. J. & Oldenbroek K. (2019). Characterization of Genetic Diversity Conserved in the Gene Bank for Dutch Cattle Breeds. Diversity. https://doi.org/10.3390/d11120229

Publications submitted for review

van Breukelen, A. E., de Haas, Y., Aldridge, M. A., Meijer, N. & Veerkamp, R. F. (2024). Genetic relationships among methane emissions from breath, dry matter intake, body weight, and milk production traits of Dutch dairy cows.

Contributions to conferences

van Breukelen, A. E., Aldridge, M. N., de Haas, Y, & Veerkamp, R. F. (2024). Genetic relationships between methane emissions, dry matter intake, bodyweight, and milk yield of dairy cows. The 75th Annual Meeting of the European Federation of Animal Science (EAAP), Florence, Italy

van Breukelen, A. E., Aldridge, M. N., de Haas, Y., Schrooten, C., Heck, J. M. L., Visker, M. H. P. W., & Veerkamp, R. F. (2024). Towards breeding for lower enteric methane emissions of dairy cows in the Netherlands. The 47th ICAR Annual Conference, Bled, Slovenia

Bonifazi, R., Flossdorf, D., Gredler-Grandl, B., Honerlagen H., Aldridge, M. N., Spoelstra, M., **van Breukelen, A. E.**, de Haas Y., & A. C. Bouwman, A.C. (2024). Impact of microbiome on genomic predictions for methane emissions in Holstein cows. The 75th Annual Meeting of the European Federation of Animal Science (EAAP), Florence, Italy

Manzanilla-Pech C. I. V., Vandenplas J., **van Breukelen A. E.**, Veerkamp R. F., & B. Gredler-Grandl (2024). Effect of heat stress on methane emissions of Dutch Holstein population. Interbull, Bled, Slovenia

Manzanilla-Pech C. I. V., **van Breukelen A. E.**, Gonzalez-Recio O., Teran E., Pszczola M., Strabel T., Veerkamp R. F., & Gredler-Grandl B. (2024). Exploring different definitions of methane concentration phenotypes in dairy cattle. The 75th Annual Meeting of the European Federation of Animal Science (EAAP), Florence, Italy

Flossdorf D., Bonifazi R., Gredler-Grandl B., Honerlagen H., Aldridge M. N., Spoelstra M., **van Breukelen A. E.**, de Haas Y., Bouwman A. C. (2024). Large scale analysis of the cattle rumen microbiome in relation to methane emissions. The 75th Annual Meeting of the European Federation of Animal Science (EAAP), Florence, Italy

van Breukelen, A. E., Veerkamp, R. F., Aldridge, M. N., & de Haas, Y. (2023). Methane emissions of dairy cows. World Holstein Friesian Federation 15th World Conference (WHFF), Puy Du Fou, France

van Breukelen, A. E., Veerkamp, R. F., Aldridge, M. N., & de Haas, Y. (2023). Genetic control of ruminant methane emissions in livestock. World Association for Animal Production session (WAAP), at the 74th Annual Meeting of the European Federation of Animal Science (EAAP), Lyon, France

van Breukelen, A. E., Veerkamp, R. F., de Haas, Y., & Aldridge, M. N. (2023). Genetic parameters for enteric CH_4 emissions of dairy cows using random regression models. The 74th Annual Meeting of the European Federation of Animal Science (EAAP), Lyon, France

Benzoni, L., Berry, D., Dressler, R., Hegarty, R., Koning, L., McDonnell, C., McNaughton, L., Ritchie, G., Finocchiaro, R., **van Breukelen, A.E.**, Garcia-Rodriguez, A., Gonzalez Recio, O., Richardson, C., Villumsen, T. M. Gredler-Grandl, B. (2023). GreenFeed and sniffer standard operating procedure (SOP) in dairy and beef cattle. The 46th ICAR Annual Conference, Toledo, Spain

van Breukelen, A. E., Aldridge, M. A., Veerkamp, R. F., Koning, L., Sebek, L.B., & de Haas, Y. (2022). Enteric methane emissions of dairy cows measured by sniffers and GreenFeed. The 12th World Congress on Genetics Applied to Livestock Production (WCGALP), Rotterdam, the Netherlands

van Breukelen, A. E., Aldridge, M. A., Veerkamp, R. F., & de Haas, Y. (2022). Using sniffers for large scale phenotyping of CH_4 emissions in the Netherlands. The Animal Selection, Genetics and Genomics Network meeting (ASGGN), at the 12th World Congress on Genetics Applied to Livestock Production (WCGALP), Rotterdam, the Netherlands

van Breukelen, A. E., Aldridge, M. A., Veerkamp, R. F., Koning, L., Sebek, L.B., & de Haas, Y. (2022). Using Spot-Sample Breath Measurements of Methane Emissions from Dairy Cattle for Genetic Evaluations. The 8th International Greenhouse Gas and Animal Agriculture Conference (GGAA), Orlando (Florida), the United States

Aldridge, M. N., **van Breukelen, A. E.**, Veerkamp, R. F., & de Haas Y. (2022). Large scale phenotyping of methane for genetic evaluation is possible with 'Sniffers'. The 45th ICAR Annual Conference, Montreal, Canada

van Breukelen, A. E., Aldridge, M. A., Veerkamp, R. F., Koning, L., Sebek, L.B., & de Haas, Y. (2022). Measurements of enteric CH_4 from two non-invasive sensors for genetic evaluations, WIAS annual conference, Lunteren, the Netherlands

van Breukelen, A. E., Aldridge, M. A., Veerkamp, R. F., Schrooten, C. & de Haas, Y. (2021). Genetic parameters for long term recording of enteric methane emissions of dairy cows. The 72nd Annual Meeting of the European Federation of Animal Science (EAAP), Davos, Switzerland

Hulsegge, I., **van Breukelen, A. E.**, Petie, R., Gonsalez, J., & Kamphuis, C. (2021). Automated area of interest and feature extraction from thermal images for cattle lameness monitoring. The 72nd Annual Meeting of the European Federation of Animal Science (EAAP), Davos, Switzerland

van Breukelen, A. E., Aldridge, M. A., Veerkamp, R. F., Schrooten, C. & de Haas, Y. (2021). Reducing enteric methane emissions of dairy cows through animal breeding techniques. Dairy Science and Technology Symposium, online

van Breukelen, A. E., Hoving, A. H., Veerkamp, R. F., & Ducro, B. J. (2021). Dairy cows enabling circular production systems. ICAR annual conference, online

van Breukelen, A. E., Aldridge, M. A., Veerkamp, R. F., Schrooten, C. & de Haas, Y. (2021). Animal breeding can be used to reduce enteric methane emissions of dairy cows. WIAS annual conference, online

van Breukelen, A. E., Aldridge, M. A., Koning, L., Sebek, L.B., & de Haas, Y. (2020). Ranking cows for methane emission measured with sniffer and GreenFeed systems. European Federation of Animal Science virtual meeting (EAAP), online

van Breukelen, A. E., Doekes, H. P., & Oldenbroek, J. K. (2019). Optimization of genomic diversity in a gene bank for Dutch cattle breeds. 70th Annual meeting for the European Federation of Animal Science (EAAP), Ghent, Belgium

Training and Supervision Plan

Education and Training	Year	ECTs*
A. The Basic Package		**2.9**
WIAS Introduction Day	2020	0.3
WGS Scientific Integrity course	2021	0.6
WGS Ethics and Animal Sciences course	2021	0.8
WIAS Introduction course on Personal Effectiveness for your PhD	2021	1.2
B. Disciplinary Competences		**14.1**
Writing the research proposal	2020	6.0
Quantitative Genetics Discussion Group	2020-2023	2.0
Genomic Prediction in Plants and Animals (Aarhus, Denmark)	2022	3.0
Genomic Prediction in Animal & Plant Breeding (Wageningen, the Netherlands)	2022	1.5
Cloud Workshop organised by Dirk Jan Schokker (online)	2021	0.1
Genomic Prediction Considering Admixed Populations and GxE (Wageningen, the Netherlands)	2022	0.9
Review two WIAS PhD proposals	2022-2023	2.0
WIAS Course Simulation of Breeding Programs with Modular Breeding Program Simulator (MoBPS) (Wageningen, the Netherlands)	2023	0.6
C. Professional Competences		**8.2**
Research Data Management	2021	0.5
Member WIAS Associated PhD Students Council and Wageningen PhD Council	2021-2022	3.0
Scientific Writing	2021	1.8
Workshop Pitch to Bewitch by Mariska Wessel (online)	2021	0.1
Workshop on Supervising MSc Students by Marieke van Schaik (online)	2021	0.1
WIAS The Final Touch: Writing the General Introduction and Discussion	2023	0.6

D. Societal Relevance		**3.5**
WIAS course Societal Impact of your Research	2021	1.5
News article "Minder methaan produceren kan ook via fokkerij"	2021	1.0
Organising and participating in yearly meetings on methane with farmers	2021-2023	1.0
E. Presentation Skills		**4.0**
EAAP, online (Oral)	2020	1.0
WIAS Annual Conference, online (Oral)	2021	
ICAR, online (Oral)	2021	
Dairy Science and Technology Symposium, online (Oral)	2021	
EAAP, Davos, Switzerland (Oral)	2021	
WIAS Annual Conference, Lunteren, the Netherlands (Poster)	2022	
GGAA, Orlando, Florida, the United States (Oral)	2022	1.0
WCGALP, Rotterdam, the Netherlands (Oral)	2022	1.0
ASGGN meeting, Rotterdam, the Netherlands (Oral)	2022	
EAAP, Lyon, France (Oral)	2023	
WAAP, Lyon, France (Oral)	2023	1.0
WHFF, Puy Du Fou, France (Oral)	2023	
ICAR, Bled, Slovenia (Oral)	2024	
EAAP, Florence, Italy	2024	
F. Teaching Competences		**6.0**
Supervising 3 MSc Major thesis students	2021-2023	4.0
Assisting course Genetic Improvement of Livestock	2021	2.0
Assisting course Animal Breeding and Genomics	2021	
Assisting course Biology of Domesticated Animals	2023	
Assisting course Introduction to Animal Sciences	2023	
Total		**38.7**

Acknowledgements

I am very grateful for all the people around me and the support I received during the last four years and would like to thank everyone who has contributed to this thesis. I would like to thank a few people in particular who have played an important role in the past four years. First, I would like to thank my supervisors Roel, Michael, Yvette, thank you for believing in me throughout the full project. It was challenging at times, but I have learned a lot in the process which I am very grateful for. Roel, thank you for all the discussions we had, especially towards the end of my thesis. Your enthusiasm about quantitative genetic analyses is contagious and made me appreciate all the modelling we did more and more. Michael, thank you for your endless optimism and being able to help me relativise the many challenges I faced during the project. I learned a lot from you as my first PhD supervisor, and hope you enjoyed having me as your first PhD student. Yvette, thank you for your trust in me during the project. Although we did not have the chance to interact much in person because of Covid and thereafter your new position, I did value your input in the project over the years and your nice words that motivated me to keep moving forward.

I am also grateful for the support I received from CRV and FrieslandCampina, and the meaningful discussions we had throughout the project. In particular, I would like to thank CRV, and especially Chris and Niek, for providing the cow data needed to do the genetic analyses. Gerben, thank you for letting me take part in the meaningful discussions towards the end on how to implement CH_4 into breeding programmes at CRV. I found it very enjoyable to get a sense of how our work is being used for practical application. Niek, thank you for the work you did on the data during the last months, and for the extra contributions to this thesis by estimating the MACE correlations. I am happy you were able to continue working on methane at CRV after your MSc thesis, so we got the chance to meet in person instead of only through a computer screen during Covid.

Very importantly, I would also like to thank Harm and Theo. Without all your hard work, driving around the Netherlands to install and maintain the sniffers, there would be no data to analyse. Although the process was often frustrating, your effort and ideas really helped me to keep moving forward. Also, thanks to Pieter and Hanne for jumping in when the work was getting over our heads and to Johan for taking over while I had to write this thesis. Without this I would have not been able to round things up so quickly. Also, a big thanks to Gerrit and Bert for creating and maintaining the data infrastructure. Without we would have not been able to process such large amounts of data so quickly. Maybe the most important of all, I would like the farmers that participated in this research. I am grateful for their hospitality and the nice conversations I had when visiting some of the farms. Even though the times are challenging to be a dairy farmer in the Netherlands, there was a great interest in participating

in this project and I really enjoyed seeing such a variety of methods implemented on farms to practise sustainable farming and enjoyed conversing about how the sector can move forward.

I would also like to thank all colleagues at ABG, who directly contributed to this thesis or just by contributing to the pleasant atmosphere in our group. Especially, I would like to thank Jan-Erik and Yuuko for making the average working day fun and always being there to discuss our PhDs and personal life. I hope that we can continue birdwatching, together with Tobi, for many more years. Also, I would like to thank my friends and colleagues that occasionally helped me forget about my PhD outside of working hours, while doing game-nights, pub quizzes, disk golf, other sports, and many more things. Torsten, Yuqi, and David, I really enjoy our bouldering sessions, and look forward to continuing them soon in Ede. I am still waiting for the day of my first 7a. Rox, I am very happy we keep in touch and can discuss all little things in life. Thank you for taking the time to read through my thesis in the last stages, when due to all the hours spent writing my head failed to grasp any logical flow and my writing became hard to understand.

Ook wil ik graag mijn familie bedanken. Pap en mam, bedankt dat jullie mij altijd steunen en voor alle kansen die ik heb gekregen in mijn leven. Dit heb ik voor een groot deel aan jullie te danken. Manon, bedankt dat ik altijd bij je terecht kan om te kletsen, hopelijk gaan we nog veel mooie plekken zien samen. Bas, bedankt voor het ontwerpen van de cover van mijn thesis, ik ben er trots op om een van jouw tekeningen hier te hebben staan er ben erg dankbaar dat je hier tijd voor wilde maken. Lieve oma, bedankt. Ik had nooit verwacht hier te eindigen, ook al wist jij het al wel. En natuurlijk Pien, Jet en Bene. Bedankt dat jullie er elke dag voor mij zijn en ervoor zorgen dat ik altijd tot rust kan komen. Jullie maken mijn huis thuis en hebben eraan bijgedragen dat ik zonder kleerscheuren (misschien met wat blauwe plekken) het einde van mijn PhD project heb bereikt.

Colophon

The research described in this thesis was financially supported by the TKI Agri & Food project LWV19155 (Wageningen, the Netherlands) and the partners CRV (Arnhem, the Netherlands) and FrieslandCampina (Amersfoort, the Netherlands)

The cover of this thesis was designed by Bas van Breukelen

Figure 1.1 and 1.3 were created with BioRender.com

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