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Abstract – Nederlands De Nederlandse overheid heeft doelen gesteld om de nationale broeikasgasuitstoot met 49% te verlagen in 2030 ten opzichte van 1990. Om dit te bereiken dient de melkveesector in 2030 de emissie van methaan (CH_4) te hebben verlaagd met 1,0 megaton CO_2-equivalenten ten opzichte van 2017. Ongeveer 80% van de CH_4 emissie van de melkveesector komt van enterisch CH_4. Het doel van deze inventarisatie was daarom: 1) Inzicht krijgen in de gemiddelde enterische CH_4 emissie en variatie van de Nederlandse melkveehouderij, 2) inventariseren welke eigenschappen hier effect op hebben, en 3) de gemeten CH_4 emissie vergelijken met de modelmatig geschatte CH_4 emissie van dat bedrijf. In totaal is de CH_4 productie met behulp van de Greenfeed (C-lock Inc.) gemeten van 791 melkkoeien (996 metingen) van 18 bedrijven verspreid door Nederland tussen september 2018 en oktober 2019. De gemiddelde CH_4 productie was 437±94 g CH_4/koe/dag en per kg meetmelk 14,4±5,1 g CH_4/kg FPCM. Uit de Lineaire-Mixed-Model analyse waarbij parameters zijn geschat met *Restricted Maximum Likelihood* (REML) bleek dat 49% van de totale variatie verklaard werd door bedrijfs- en diereigenschappen: grondsoort (6%), grazen in relatie tot het seizoen (3%), lactatiestadium en pariteit (32%), het gehalte ureum en lactose in de melk en de lactatiewaarde (samen 8%). Rantsoeneigenschappen leverden geen substantiële bijdrage aan de verklaring van de geobserveerde variatie in CH_4 emissie. Er werd geen correlatie gevonden tussen de gemeten en modelmatig geschatte gemiddelde CH_4 emissie op bedrijfsniveau. Vervolgonderzoek is nodig voor het verbeteren van modelberekeningen en om het effect van vers gras, vers gras kwaliteit, het microbioom in de pens en genetica op CH_4 emissie te kwantificeren.

Abstract – English The Netherlands aims to reduce greenhouse gas emissions by 49% in 2030 compared to 1990. In order to achieve this goal the dairy sector needs to reduce methane (CH_4) emissions by 1.0 megaton CO_2-equivalents compared to 2017. Approximately 80% of the CH_4 emission of the dairy sector originates from enteric CH_4. The objectives of this study were therefore: 1) To gain insight into the average enteric CH_4 emission and variation of the Dutch dairy herd, 2) to investigate which factors have an influence on the variation, and 3) to compare the measured CH_4 emission per farm to the estimated emission using model calculations. In total CH_4 production was successfully measured from 791 dairy cows (996 records) of 18 farms throughout the Netherlands for a period of 2 weeks from September 2018 to October 2019 using Greenfeed (C-lock Inc.). The average CH_4 production was 437±94 g CH_4/cow/day and per kg fat-protein corrected milk 14.4±5.1 g CH_4/kg FPCM. According to the Linear Mixed Model analysis fitted with Restricted Maximum Likelihood 49% of the total variation was explained by farm and animal factors: soil type (6%), grazing related to season (3%), lactation stage and parity (32%), the content of urea and lactose in the milk and the lactation value (together 8%). Feed composition and feed quality components did not show a significant effect on the observed variation. A comparison of the average herd emission of single farms revealed no correlations between the CH_4 emission measured in this inventory and estimated using model calculations. Further research is required on the effects of fresh grass as well as fresh grass quality, rumen microbiome or genetics on CH_4 emission.

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Foreword

The long-term global climate goals of the Paris Agreement, adopted by nearly 200 countries in December 2015, imply the necessity for enhanced efforts for greenhouse gas emissions reductions in the Netherlands. Compared to the reference year 1990, the Dutch greenhouse gas (GHG) emission has to decrease with 49% by 2030 and 95% by 2050. The translation of the goals of the international climate regime into the context of the Netherlands was based on the comparison of different emissions reduction scenarios. These scenario's included reduction goals for the dairy sector and revealed that reducing methane (CH_4) emissions is important. In order to establish achievable reduction goals for the Dutch dairy farmers information was needed of emission level and variation. However, data of CH_4 emission on commercial dairy farms were not available. These data were generated and the results are presented in this report. The study described is an extensive inventory within the Dutch dairy herd to gain insight in emission level and variation of enteric methane emission.

This research was conducted by Wageningen Livestock Research and commissioned and funded by the Dutch Ministry of Agriculture, Nature and Food Quality. The data were collected in collaboration with the project 'Cows and Opportunities', a network of 16 practical farms and the research farms 'De Marke' and 'KTC Zegveld'. Together they represent the Dutch national dairy herd.

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Samenvatting

In het klimaatakkoord dat Nederland in 2015 tekende staan doelen beschreven voor de reductie van broeikasgassen om de opwarming van de aarde tegen te gaan. De Nederlandse overheid heeft als doel gesteld om de nationale broeikasgasuitstoot met 49% te verlagen in 2030 ten opzichte van het referentiejaar 1990. Om dit te bereiken zijn er voor verschillende sectoren specifieke doelen gesteld. De melkveesector dient in 2030 de emissie van methaan (CH_4) te verminderen met 1,0 megaton CO_2-equivalenten ten opzichte van 2017. Ongeveer 80% van de CH_4 emissie van de melkveesector komt van enterisch CH_4; CH_4 dat in de koe wordt gevormd door de fermentatie van (ruw)voer en voornamelijk via de bek wordt uitgescheiden. De reductie van enterisch CH_4 is daarom van groot belang.

Volgens het *National Inventory Report* van 2019 was de CH_4 productie van de Nederlandse melkveesector in 2017 (tijdens lactatie) 401 g CH_4 per koe per dag en 13.5 g CH_4 per kg meetmelk (FPCM, melk voor vet- en eiwit gecorrigeerd). De informatie over de gemiddelde CH_4 productie en de variatie van emissie in de Nederlandse melkveesector komt van modelmatige schattingen met behulp van een internationaal geaccepteerd model. Dat model is gebaseerd op data van experimenten in respiratiekamers. De invoer van het model bestaat uit praktijkdata met betrekking tot melkproductie, rantsoensamenstelling (voedermiddelen) en voeropname. Het is echter onbekend of het model de variatie van de Nederlandse melkveesector goed weergeeft.

De onderzoeksdoelen van deze inventarisatiestudie waren daarom:

1. Inzicht krijgen in de gemiddelde CH_4 emissie en variatie van de Nederlandse melkveehouderij.
2. Inventariseren of bedrijfs- en/of diereigenschappen effect hebben op de CH_4 emissie en variatie.
3. Vergelijken van de gemeten CH_4 emissie met de modelmatig geschatte CH_4 emissie van dat bedrijf.

De enterische CH_4 productie is op praktijkbedrijven gemeten met behulp van de Greenfeed (C-lock Inc.), een aangepast krachtvoerstation dat zowel de CH_4 concentratie meet als de kwantitatieve luchtstroom. Daarnaast registreert de Greenfeed de positie van de kop van de koe waarmee wordt bepaald of er een correct monster wordt gemeten. Deze data zijn gebruikt om de kwantitatieve CH_4 productie (in gram per koe per dag) per bezoek te berekenen. In totaal is de CH_4 productie succesvol gemeten van 791 melkkoeien (996 metingen) van 18 bedrijven verspreid door Nederland. Op elk bedrijf is gedurende een periode van twee weken gemeten (plus één week adaptatie) die lag tussen september 2018 en oktober 2019. Per bedrijf is de individuele melkdata verzameld en op groepsniveau de voeropname inclusief voersamenstelling en -kwaliteit. De data is geanalyseerd met een Lineaire-Mixed-Model analyse waarbij parameters zijn geschat met *Restricted Maximum Likelihood* (REML). De belangrijkste resultaten zijn:

1. De gemiddelde CH_4 productie was 437±94 g CH_4/koe/dag en de CH_4 intensiteit (CH_4 productie per kg meetmelk) was 14,4±5,1 g CH_4/kg FPCM.
2. De geobserveerde variatie (standaard deviatie) was met 94 g CH_4/koe/dag en 5,1 g CH_4/kg FPCM groot.
3. In totaal kon circa 49% van de totale variatie worden verklaard: 9% door bedrijfs- en 40% door diereigenschappen. De bedrijfseigenschappen waren grondsoort waarop het bedrijf zich bevond (klei, veen, zand) (6%) en wel of niet weiden afhankelijk van het seizoen (3%). De diereigenschappen waren lactatiestadium en pariteit (samen 32%) en het gehalte ureum en lactose in de melk en de lactatiewaarde (samen 8%).
4. Lactatiestadium en pariteit bepaalden voor het grootste deel de variatie, circa 32%. Dit was voornamelijk binnen-koe variatie (binnen koeien over meetperioden, dus over de tijd).
5. Rantsoeneigenschappen leverden geen substantiële bijdrage aan de verklaring van de geobserveerde variatie in CH_4 intensiteit. Hoewel grondsoort en beweiden afhankelijk van

seizoen een deel van de variatie verklaarde, waren deze factoren niet te vervangen door rantsoeneigenschappen zoals het percentage snijmaissilage of vers gras in het rantsoen.

6. Er is geen correlatie gevonden tussen de gemeten CH_4 intensiteit en de modelmatig berekende CH_4 intensiteit op basis van rantsoeneigenschappen. Dit suggereert dat het gebruikte theoretische model onvoldoende gevalideerd is voor gebruik in de praktijk op bedrijfsniveau. Op nationaal niveau is de schatting van het model wel goed.

De gemeten enterische CH_4 productie in de praktijk komt overeen met de modelmatig geschatte landelijke productie beschreven in het *National Inventory Report* van 2019. De grote variatie in CH_4 productie werd verwacht. Het betreft de variatie tussen alle koeien van alle bedrijven en metingen, dus over verschillende systemen en rantsoenen. De meeste variatie was zichtbaar op koe niveau (tussen koeien binnen een bedrijf en binnen een koe over meetperioden) vergeleken met bedrijfsniveau (tussen bedrijven en binnen een bedrijf over meetperioden). Een restvariatie van circa 50% kon niet verklaard worden door de bedrijfs- en diereigenschappen die meegenomen zijn in deze inventarisatie. Vervolgonderzoek is nodig om te kwantificeren welke andere eigenschappen effect kunnen hebben op de emissie, zoals de werking en de verhouding van microben in de pens of genetica.

Een mogelijke verklaring voor het grote effect van lactatiestadium en pariteit op de variatie is dat koeien vroeg in de lactatie in een negatieve energiebalans zitten, zij geven meer melk dan ze aan de energie opnemen. Daarnaast krijgen koeien vroeg in de lactatie meer krachtvoer ten opzichte van koeien later in de lactatie, waardoor de CH_4 intensiteit lager is. Vaarzen stoppen een deel van hun energieopname in groei, waardoor ze naar verhouding met oudere koeien per kg meetmelk een hogere CH_4 productie hebben.

De grondsoort waar het bedrijf op staat verklaarde een groot deel van de tussenbedrijfsvariatie. Er zijn geen bewijzen gevonden dat dit afhankelijk was van het rantsoen. Geen van de rantsoeneigenschappen had een significant effect op de CH_4 intensiteit. Enkel wel of niet beweiden afhankelijk van het seizoen (zomer: maart tot en met augustus, winter: september tot en met februari) had effect op de variatie. Vers gras gaf mogelijk een lagere CH_4 productie. Vervolgonderzoek om het effect van vers gras en vers gras kwaliteit op emissies nader te onderzoeken wordt aanbevolen.

Tot slot werd er geen correlatie gevonden tussen de gemeten en modelmatig geschatte gemiddelde CH_4 intensiteit en productie op bedrijfsniveau. Wel was de grootte van de afwijking tussen het model en de gemeten emissie afhankelijk van het bedrijf. In de praktijk komt een grote verscheidenheid aan rantsoenen en voerstrategieën voor die buiten het betrouwbaarheidsinterval van het model vallen. Voor niet reguliere rantsoenen voorspelt het model de CH_4 productie mogelijk onvoldoende. Vervolg op deze inventarisatie zal zich richten op voerstrategieën voor de praktijk met als doel zowel CH_4 als ammoniak te reduceren en de gebruikte modellen om CH_4 productie te schatten te valideren in de praktijk.

Summary

Resulting from the Paris Climate Agreement of December 2015, the Netherlands aims to reduce greenhouse gas (GHG) emissions by 49% in 2030 compared to the reference year 1990. To achieve this goal the dairy sector needs to reduce methane (CH_4) emissions by 1.0 megaton CO_2-equivalents compared to 2017. Approximately 80% of the CH_4 emissions of the dairy sector originates from enteric CH_4. Therefore, reduction of enteric CH_4 emission of dairy cows is an important issue.

The average enteric CH_4 production of lactating dairy cows was, according to the National Inventory Report of 2019, 401 g CH_4/cow/day and 13.5 g CH_4/kg FPCM (fat and protein corrected milk) in 2017. The average CH_4 emission and variation of dairy cows in practice are currently estimated using model calculations which are internationally accepted (e.g. IPCC Tier 3). This model is based on data of respiration chambers. It is however unknown if the average emission and variation measured in experimental research is representative for what is seen in practice. It is uncertain if the model estimates the variation in the Dutch dairy sector correctly.

The objectives of this study were therefore:

1. To gain insight into the average CH_4 emission and variation of the Dutch dairy herd.
2. To investigate which farm and animal factors were related to the enteric CH_4 emission.
3. To compare the measured emissions per farm with the estimated emissions using model calculations.

Enteric CH_4 production was measured using the Greenfeed (C-lock Inc.), an adapted feeding station that measures both continuously CH_4 concentration and the quantitative airflow. The Greenfeed also takes into account head positioning for correct measurements. These parameters were used to quantify the CH_4 flux caused by the animal during each visit. CH_4 concentration was analysed by a non-dispersing infrared analyser. In total 791 dairy cows (996 records) of 18 farms throughout the Netherlands were successfully measured for a period of two weeks (plus one week of adaptation) from September 2018 to October 2019. Per farm individual milk samples were taken to analyse milk composition. Feed intake data were collected on herd level (including feed composition and quality). Linear Mixed Models fitted with Restricted Maximum Likelihood were used to analyse the data. The most striking results were:

1. The average CH_4 production was 437±94 g CH_4/cow/day and the CH_4 intensity (CH_4 production per kg measuring milk) 14.4±5.1 g CH_4/kg FPCM.
2. The observed variation (standard deviation of 94 g CH_4/cow/day and 5.1 g CH_4/kg FPCM) was large.
3. Around 49% of the total variation is explained by factors as farm (9%) and animal (40%). Farm factors included soil type of the region the farm is located (sand, clay, peat, 6%) and grazing related to season (3%). Animal factors included lactation stage and parity (32%), and the content of urea and lactose of the milk and the lactation value (8%).
4. Lactation stage and parity explained the largest part of the variation, approximately 32%. This is mainly within-cow variation (within a cow over measurement periods, *i.e.* over time).
5. Feed composition and feed quality components did not have a significant effect on the observed variation. Although soil type and grazing related to season did explain part of the variation, these factors could not be replaced by more specific factors like the percentage of maize or fresh grass in the diet.
6. No correlations were found between the measured CH_4 intensity and the calculated CH_4 intensity using model calculations. This suggests that the model calculations can only estimate the CH_4 emission within a limited range on farm level. Nationally the model estimates the emission sufficient.

The measured enteric CH_4 production was comparable to the estimated production based on model calculations described in the National Inventory Report of 2019. The large observed variation was

expected. It is the variation between all cows of all farms throughout the year, with different management systems, productivity and feed compositions. Most variation is seen on cow level (between cows of a farm and within a cow over measurement periods) rather than farm level (between farms and within a farm over measurement periods). Around 50% of the variation could not be explained by the farm and animal factors taken into account within this inventory. Further research is required to investigate the effect of other characteristics, like the composition and functioning of the rumen microbiome or genetics.

Lactation stage and parity had the largest observed effect on the variation. A possible explanation is the negative energy balance of cows in early lactation. The production rate of cows in early lactation is higher than the energy intake. Cows in early in lactation also receive more compound feed, resulting in a lower CH_4 intensity than cows in late lactation. Heifers use part of their energy intake for growth instead of production, resulting in a higher CH_4 intensity compared to older cows.

Soil type of the location of the farm explained most of the variation between farms. No evidence was found that this was caused by diet composition. No effect of the percentage of maize silage nor other feed characteristics was found on CH_4 intensity. Whether or not cows were grazing related to season (summer: March to August, or winter: September to February) did show a small effect on CH_4 intensity. The effect of fresh grass as well as fresh grass quality should be further investigated in an experimental study.

At last, the comparison of the average herd emission of single farms revealed no correlations between the CH_4 intensity and production measured in this inventory versus estimated using model calculations. There was however an effect of farm on the difference between CH_4 measurements and model calculations. For some farms the difference was considerably larger than for other farms, suggesting that the model has difficulty estimating corresponding CH_4 emission of certain diets. Follow up research of this inventory will focus on feeding strategies feasible in practice that aim to reduce both CH_4 and ammonia emissions.

1 Introduction

Resulting from the Paris Climate Agreement of December 2015, the Netherlands aims to reduce greenhouse gas (GHG) emissions by 49% from 227 million tons (MT) carbon dioxide equivalents (CO_2-eq.) in 1990 to 116 MT CO_2-eq. in 2030 (Coenen *et al.,* 2017). In 2017 total Dutch GHG emission was 197 MT CO_2-eq. (figure 1.1). The prospect is that due to the current climate policy this will decrease to 165 MT CO_2-eq. in 2030. Main causes for the reduction achieved in 2017 compared to 1990 relate to a higher efficiency of electricity and other sources, like the decrease of fuel per kilometre and the increase of electricity generation from wind. Finally, a significant decrease in emission, specifically of methane (CH_4), is seen because of improvements in the waste disposal sector (CBS *et al.,* 2017).

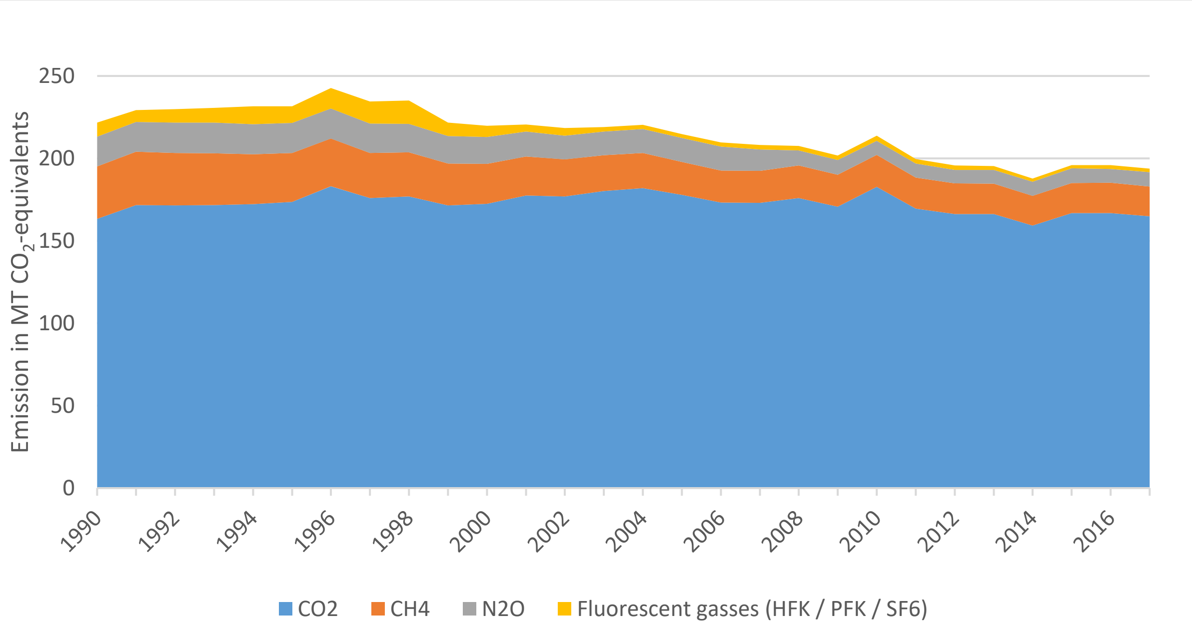


Figure 1.1 *Overview of Dutch GHG emission of CO_2 (carbon dioxide), CH_4 (methane), N_2O (nitrous oxide) and fluorescent gasses in MT CO_2-eq. from 1990 to 2017. Data from CBS (CBS et al., 2017).*

To further reduce the GHG emission from 197 MT CO_2-eq. in 2017 to 116 MT CO_2-eq. in 2030, specific goals have been made for different sectors (figure 1.2). The Dutch agricultural sector produces approximately 10% of the total Dutch GHG emissions (19.2 MT in 2015; Coenen *et al.,* 2017), and has been assigned to reduce 3.5 MT CO_2-eq. by 2030. The livestock sector, specifically the dairy sector, has to reduce CH_4 emissions by 1.0 MT CO_2-eq. Approximately 80% of the CH_4 emissions of the dairy sector originates from enteric CH_4. Reduction of the enteric CH_4 emission of dairy cows is therefore an important issue.

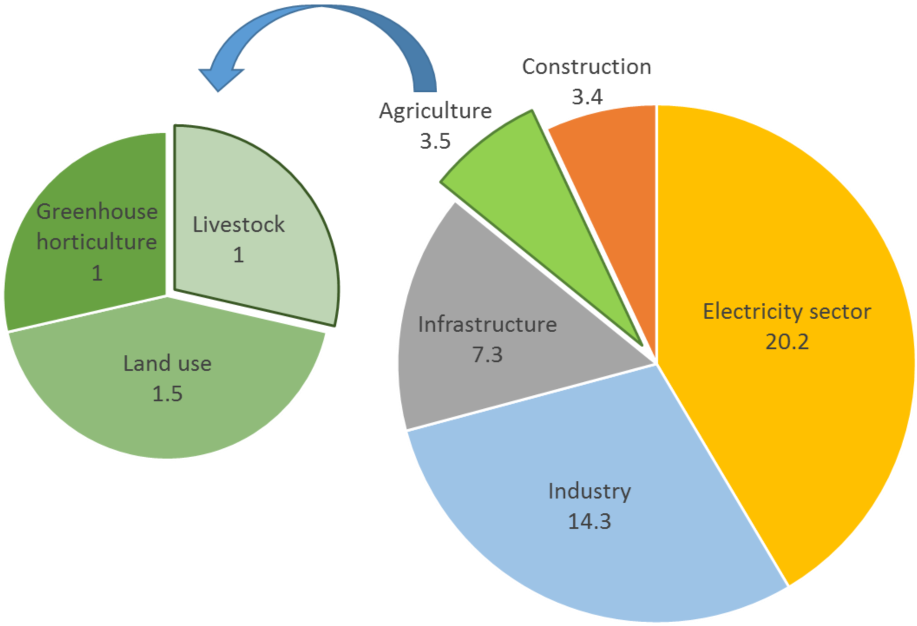


***Figure 1.2** Schematic representation of the Dutch GHG reduction aim for different economic sectors, to reduce in total 49 MT CO_2-eq from 2017 to 2030 (additional to the current climate policy). The right pie chart depicts the five sectors and their corresponding goals to reduce their emissions in MT CO_2-eq. The left pie chart specifies the reduction aim of three agricultural sectors, of which the livestock has to reduce their emissions by 1 MT CO_2-eq. Data from the Dutch Climate Agreement (Klimaatakkoord 2019).*

This report describes an inventory study that gained insight into the average CH_4 emission and variation of the Dutch dairy herd. It investigated factors that might affect this emission and variation. Finally, the measured emissions were compared to model calculations used by the Dutch Inventory (see chapter 2).

In chapter 2 a literature background of the currently used model to estimate CH_4 emission and possible reduction potentials is presented. The chapter ends with the objectives of this report. In chapter 3 the materials and methods are described. An overview of the results of this inventory study is given in chapter 4, including the average CH_4 emission and variation of the Dutch dairy herd and factors that might affect the emission. The results are discussed in chapter 5 and conclusions are made.

2 Background

2.1 Emission according to the Dutch Inventory

The Dutch protocol for the National Inventory of agricultural GHG emissions includes a dynamic and mechanistic model to estimate enteric CH_4 emission by dairy cows (Bannink *et al*., 2011). This model is an approved Intergovernmental Panel on Climate Change (IPCC) Tier 3 approach and was developed on extensive experimental data from climate respiration chambers. According to this model the enteric CH_4 production of the Dutch dairy sector (lactating cows) was 5.2 MT CO_2-eq. in 1990 and 5.6 MT CO_2-eq. in 2017 (Ruyssenaars *et al.,* 2019). Expressed per cow this was 302 g CH_4/cow/day in 1990 and 369 g CH_4/cow/day in 2017. Although the total emission per cow increased, production rates also increased from 1990 to 2017, resulting in a lower CH_4 intensity (CH_4 production per kg fat-protein corrected milk, FPCM). The CH_4 intensity was 18.4 g CH_4/kg FPCM in 1990 and 15.5 g CH_4/kg FPCM in 2017. The data is from year round, including the dry period where CH_4 is emitted but no milk is produced. Focussing solely on the lactation period, the production was 401 g CH_4/cow/day and the intensity 13.5 g CH_4/kg FPCM in 2017. This model can accurately estimate average enteric CH_4 emission on national level using a reference diet, but is less reliable on farm or individual level. Not every diet that is used on commercial dairy farms is extensively tested and fitted into the model. For application on farm level, the model is adapted and simplified and described by Sebek *et al.* (2016). This model calculates CH_4 based on feed composition and intake using Tier 3 estimated emissions for each feed component (emission factor (EF) in g CH_4/kg DM). EFs are corrected for diet composition, total feed intake, roughage quality (NDF and starch content) and compound feed composition (crude protein content). It is currently used in practice to estimate CH_4 emission per farm, but if it estimates the variation within the Dutch dairy herd correctly is unknown. Measurements in practice are needed to serve as a validation for the model.

2.2 Reduction potentials

Reduction potentials based on respiration chamber studies are calculated up to 60% (Haisan *et al.,* 2014). The main strategies are changing feed composition, adding feed supplements and improving farm management. All these reduction potentials focus on at least one of the following aspects of methanogenesis (as described more in detail by McAllister and Newbold (2008)):

1. Inhibition of methanogens by redirecting H_2 into alternative products
2. Decrease the production of H_2 in the rumen
3. Provide an alternative sink for H_2 disposal in the rumen

Feed composition strategies are mainly focused on shifting fermentation of fibre to fermentation of starch. Starch is digested in the small intestines without CH_4 formation. This is decreasing the production of H_2 in the rumen. An example is replacing roughage with compound feed, which reduces enteric CH_4 production with 3 to 12% (Lovett *et al.,* 2006). Based on model calculations, increasing compound feed from 338 kg to 1403 kg per cow per year decreased enteric CH_4 production from high yielding cows from 171 Mg CO_2-eq. per year to 151 Mg CO_2-eq. There is an optimum in the amount of compound feed regarding CH_4 emission and most farms in the Netherlands already feed conform this optimum.

Feed supplements can work on different pathways in CH_4 reduction. For example, the additive 3-nitrooxypropanol (3-NOP) specifically inhibits the enzyme methyl-coenzyme M reductase, which catalyses the last step in the CH_4 forming pathway of methanogens (Dijkstra *et al.,* 2018). The additive reduces enteric CH_4 emission significantly, studies report reductions of 10% (Reynolds *et al.,* 2014) to 60% (Haisan *et al.,* 2014). This additive is currently still tested on safety and efficacy and not used on commercial dairy farms yet. Whether or not these reduction potentials are also feasible in practice for both high and low producing cows and for different diets is yet to be determined.

For the Dutch dairy sector maximum reduction potentials by feeding strategies are estimated up to 37% compared to 1990, approximately 5% was already achieved in 2013 (Sebek *et al.,* 2014). A recent literature review by Van Gastelen *et al.* (2019b) gives an overview of 94 studies of dietary strategies to mitigate enteric CH_4 in either dairy cattle, beef cattle or sheep.

2.3 Objectives

Enteric CH_4 emission of the Dutch dairy herd had not been measured previously. The first objective of this study served as an inventory to gain insight into the average CH_4 emission and variation of the Dutch dairy herd. The second objective was to investigate whether or not variation in enteric CH_4 emission was affected by farm and/or animal characteristics. Thirdly, the measured emissions were compared to estimated emissions using model calculations described by Šebek *et al.* (2016) based on feed intake, composition and quality. This served as an evaluation of the model in practice.

3 Materials and methods

3.1 Animals, management, feed and milk data collection

In total the CH_4 production of 1278 dairy cows, mostly Holstein-Frisian, on 18 farms was measured for a period of 2 weeks with at least one week of habituation. Farms were measured multiple times. Data was collected from September 10th 2018 to October 14th 2019. The data originated from commercial dairy farms throughout the Netherlands. Farms were selected from "*Koeien & Kansen*" (Cows & Opportunities), a multi annual research and demonstration project designed to be representative for the Dutch dairy sector. Of each farm a group of 40 to 80 cows representative for the whole herd was selected. This selection was not completely constant over measuring periods. In this inventory study no experimental setup was applied. No changes in farm management nor feed composition were made. The obtained dataset was checked for representation for the Dutch dairy sector on lactation number, days in lactation, milk yield and average diet. During the two measuring weeks, the farmer recorded feed intake on group level (all lactating cows) and feed composition according to the protocol known within the Cows & Opportunities project. In short, all feeds were weighed before given to the cows and residuals were weighed to calculate daily feed intake of the lactating herd. Fresh grass intake was estimated (if applicable) based on the feed unit of lactation (VEM) system. The energy intake of all feed components were subtracted from the energy need to calculate fresh grass intake. Feed samples were taken once during the two week measuring period. Feed samples were analysed for chemical composition and nutritional value. Compound feed analysis was requested from the feed supplier. Milk samples were taken in the second measuring week according to routine milk-recording programs.

3.2 Feed and milk chemical analysis

Feed samples were analysed by Eurofins Agro (Wageningen, the Netherlands) using their standard protocols conform NEN-EN-ISO 17025. Dry matter (DM) was determined by gravimetry. Crude protein (RE), digestible crude protein (VRE), phosphorus, neutral detergent fibre (NDF) and starch (for maize silages) content were determined by near-infrared spectrometry (NIRS). Nitrogen (N), VEM, digestible true protein (DVE) and degraded protein balance (OEB) were calculated based on NIRS of nitrate, VRE, NDF, and lactic acid. If feed samples were not present, NDF and starch content of the complete silage batch (cut) determined by NIRS were used. For the complete list of analysed feed components see appendix 1.

Milk samples were analysed by Fourier transform mid-infrared spectrometry (MIRS) within routine milk-recording programs, predominantly performed by Qlip (Zutphen, the Netherlands). Milk yield and percentage of protein and fat in the milk of one sampling day were used to calculate FPCM via the following formula:

$$FPCM\ (kg/day) = (0.337 + 0.16 * \%fat + 0.06 * \%protein) * milk\ yield\ (kg/day)$$

3.3 CH4 emission monitoring

Enteric CH_4 production was measured non-invasively using the GreenFeed system (GF, C-lock Inc. Rapid City, SD, USA[1]). The Greenfeed is an adapted feeding station that continuously measures both CH_4 and CO_2 concentration and the quantitative airflow. It also takes into account head positioning to ensure sample quality. These parameters were used to quantify the CH_4 and CO_2 fluxes caused by the

[1] https://www.c-lockinc.com/shop/automated-emissions-measurement/GF-large-animals/

animal during each visit. CH_4 and CO_2 concentration were analysed by a non-dispersing infrared analyser, as described by Manafiazar *et al.* (2016). Cows either got their complete compound feed allowance via the GF or partly, depending on the availability of a regular feeding station. Cows received 2 to 8 kg compound feed via the GF per day, depending on their milk yield and lactation stage. The feed gift was divided over 4 to 6 feeding periods with at least 3 to 4 hours between each feeding period. Per feeding period 500 to 1400 grams of compound feed was provided by the GF in 10-15 drops of approximately 40 grams of feed. Between each drop was 10-30 seconds (depending on the maximum number of drops) to assure a minimal visit time of 3 minutes, but no longer than 6 minutes. The remaining compound feed allowance was provided via the regular feeding station (if applicable) or during milking. A more detailed description of the general operation of the GF is provided by Velazco *et al.* (2017). Calculations of the volumetric flow rates of gases, of the volumetric airflow rate and eventually the mass flux calculations are described by Huhtanen *et al.* (2015).

To receive sufficient CH_4 measurements throughout the day, different strategies were applied depending on the situation of the farm and season. When cows were fulltime at the barn, one GF was installed in the barn. At two farms two GF's were installed to measure more cows or different groups. When cows had fulltime access to the barn during pasture season, for example farms that used an automatic milking system (AMS), one GF was installed in the barn. When cows were not able to visit the barn during grazing, two GF's were used, one in the barn and one on a pasture trailer. Measurements in the pasture were corrected for wind speed using the wind speed sensor of the GF.

CH_4 measurements of the two week measuring period were averaged per cow. As studied in beef cattle by Manafiazar *et al.* (2016) and Arthur *et al.* (2017), the variation decreases when more records per animal are used. The CH_4 production of cows vary significantly over the day. In order to get a good representation of the average CH_4 production using short-term breath measures, records of multiple times per day of multiple days need to be averaged to increase reliability. Therefore, cows with less than 20 valid records were excluded from the analysis.

3.4 Estimated individual feed intake

Average individual feed intake of the test group was estimated via the recorded feed intake on herd level and *Koemodel* (Zom, 2014). *Koemodel* estimates individual feed intake based on lactation stage and parity. DMI of each cow of the complete herd as well as of the test group was calculated using *Koemodel*. Based on the difference between estimated and measured average DMI of the herd a correction was applied for the test group according to the following calculation on cow level:

$$Estimated\ DMI_{test} = FIC_{test} * \frac{DMI_{herd}}{FIC_{\ herd}}$$

With:

- DMI_{test} = the predicted average DMI in kg of the test group
- FIC_{test} = the average feed intake capacity of the test group
- FIC_{herd} = the average feed intake capacity of the herd
- DMI_{herd} = the recorded average DMI in kg of the herd

FIC was calculated according to the *Koemodel* calculation:

$$FIC = \left(8.08 + 3.2956 * \left(1 - e^{-1.2758*((LNR-1)+\frac{LD}{365})}\right)\right) * e^{0.3983*(1-e^{-0.05341*LD})}$$

With LNR the lactation number (parity) and LD the number of days in lactation.

3.5 Statistical analysis

3.5.1 Inventory of the variation observed

Statistical analysis was done on CH_4 intensity and conducted using Genstat 19th edition (version 19.1.0.21390, VSN International, Hemel Hempstead, UK). Linear Mixed Models (LMM) were used to estimate each variance component to indicate which fixed effects had influence on these variance components. Four different components (random effects) were defined: between-farm variation, within-farm variation (over measurement periods), between-cow variation (within farms) and within-cow variation (over measurement periods). A schematic representation of these variance components is shown in figure 3.1.

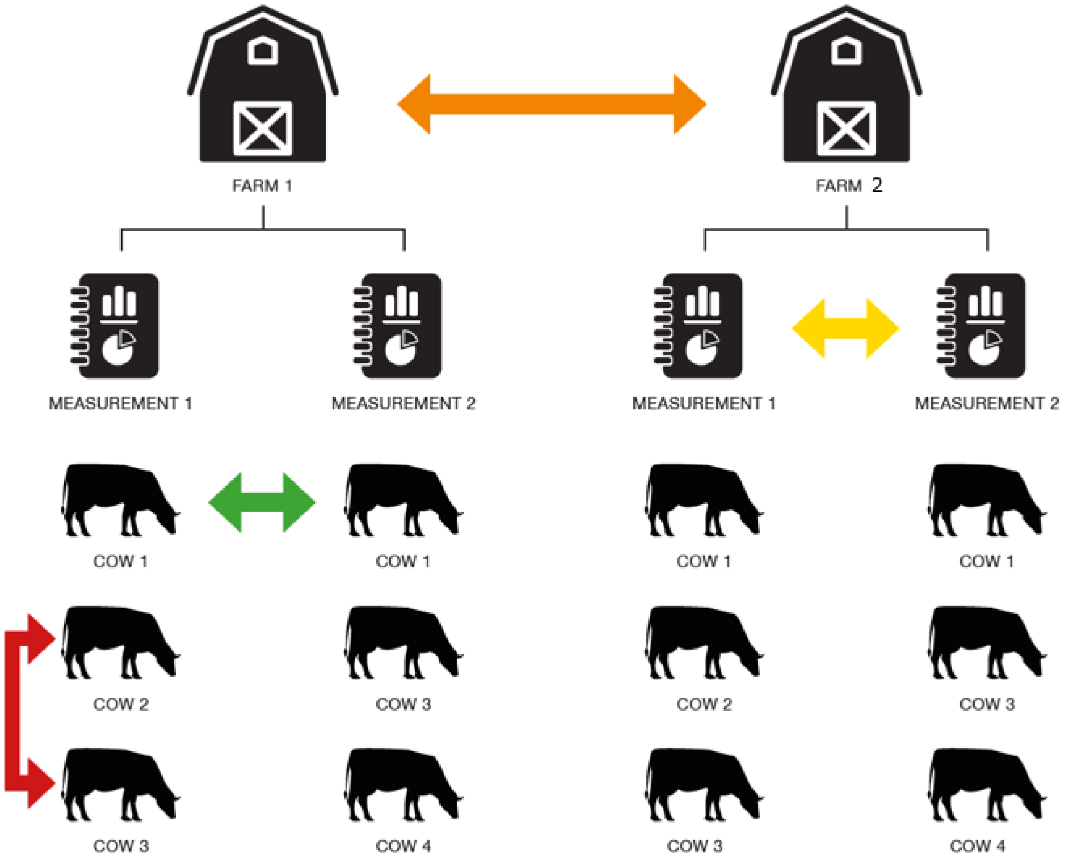


Figure 3.1 *Schematic representation of the different variance components (random effects). The arrows show the variance components: between-farm variation (orange), within-farm variation (yellow), between-cow variation (red) and within-cow variation (green). Note that not all the same cows were measured over measurement periods.*

LMM fitted with Restricted Maximum Likelihood (REML) were used to understand how the variation in enteric CH_4 intensity is distributed in practice by estimating the four variance components. The basic model (without fixed effects) with the four random effects is:

$$Y_{xik} = \mu + FARM_x + FARM_x.MEASUREMENT_i + FARM_x.COW_k + FARM_x.COW_k.MEASUREMENT_i$$

With Y_{xik} as CH_4 intensity in log-transformed g CH_4/kg FPCM for farm x (n=18), measurement period i (1, 2 or 3) and cow k (n=1278). FARM shows between-farm variation, FARM.MEASUREMENT shows within-farm variation over measurement periods (each farm was measured multiple times), FARM.COW shows between-cow variation within a farm and FARM.COW.MEASUREMENT shows within-cow variation over measurement periods (*i.e.* variation of the same cow over measurement 1, 2 and/or 3). Not all cows were measured over measurement periods.

3.5.2 Inventory of the fixed effects

To indicate which type of fixed effects could reduce the variance components, the basic REML analysis was extended with several fixed effects. The fixed effects added to the model were year (2018/2019), season (summer/winter: March to August versus September to February), grazing (yes/no), soil type of the location of the farm (sand/clay/peat), days in lactation (lactation stage), lactation number (parity) and milk production information (including composition) (appendix 1). The fixed effects relate to farm, season, animal or milk factors. Only fixed effects with an significant effect in explaining the observed variation, had little or no correlation with other effects and had some sort of biological relevance were added in the final model. Factors were added to the model in order of the degree of collection complexity:

- Region information is nationally recognized, only the location of the farm must be known.
- Seasonal information included whether or not cows were grazing, which is farm dependent and must be requested.
- For animal information like lactation stage and parity current herd information need to be requested.
- For milk information milk samples must be taken.

Partial unadjusted R^2 of fixed effects were calculated as the difference of the R^2 of two models (with and without the specific fixed effect(s)). Unadjusted R^2 was calculated via the following formula:

$$R^2 = \left(1 - \frac{\sigma_{new}^2}{\sigma_{standard}^2}\right) * 100\%$$

With σ^2_{new} the variance component estimate of the model with the fixed effect(s) added, and $\sigma^2_{standard}$ the variance component estimate of the model without any fixed effects.

3.5.3 Inventory of the effect of feed

Model outcomes of the REML analysis of the inventory of the fixed effects were used in the REML analysis for the effect of feed. Feed data was collected on group level (n=37), consequently the variance components on animal level (between- and within-cow) were not available. The effects of feed characteristics were investigated with the estimated variance components between-farm and within-farm (over measurements) from the model outcome (as described in 3.5.1. and 3.5.2). A new dataset of 37 records was made, representing all measurement periods of the farms over time. The model was corrected for animal characteristics that had a significant effect according to the analysis described in 3.5.2, to ensure effects found related to feed rather than animal characteristics like lactation stage.

In total 23 feed characteristics were collected (appendix 1). All subset regressions were performed to select feed characteristics with a significant linear relationship with the CH_4 intensity. The CH_4 Intensity was expressed as the estimated total of between- and within-farm variation log-transformed. Feed characteristics that were either significant ($p<0.05$) or a trend ($0.05<p<0.1$) were selected for the REML analysis. Feed components that were expected to show an effect were added to the model as well: percentage of maize silage, fresh grass, compound feed and the estimated DMI.

3.5.4 Evaluation of model calculations

Enteric CH_4 intensity was calculated using *Koemodel* and CH_4 model calculations. *Koemodel* was used to estimate DMI as described in chapter 3.4. Based on the estimated DMI of each cow an estimated CH_4 intensity was calculated according to the model calculations described by Sebek *et al*. (2016). In short, this model is a simplification of the Dutch Tier 3 model (Bannink *et al.*, 2011) in order to enable practical application. The model calculates CH_4 based on feed composition and intake using EF corrected for diet composition, total DMI, roughage quality (NDF and starch content) and compound feed composition (crude protein content). The calculated CH_4 intensity was compared with the measured CH_4 intensity on group level by a lack of fit analysis: a regression analysis of the predicted CH_4 intensity versus the measured CH_4 intensity.

4 Results

4.1 Representativeness of the data

This study served as an inventory for the Dutch dairy sector. Emission rates are dependent on production and DMI, so the collected dataset should be representative for the Dutch dairy herd before conclusions can be drawn. The distribution of parity, lactation stage, milk yield and diet composition (including DMI) of the dataset was therefore compared with Dutch annual statistics (table 4.1).

In 2018 the average distribution of parity in the Netherlands was 33%, 27% and 40% respectively for heifers, second and higher than second lactation cows (table 4.1; CRV, 2019). Lactation stage was approximately equally distributed on a yearly basis. Average milk production of the Dutch herd was 29.7 kg FPCM/cow/day with a DMI of 20.3 kg (CRV, 2019). Parity and lactation stage were similarly distributed in the dataset of the test group (table 4.1). Milk yield and DMI was slightly higher in the test group, 32.4 kg FPCM and 21.5 kg DM, compared to annual Dutch averages, but within standard deviation (SD[2]) ranges (appendix 1). Average diet composition is also comparable between the Dutch annual statistics (data from 2017; CBS, 2018) and the dataset: diets consisted of approximately 35% grass silage, 20% maize silage, 15% fresh grass, 25% compound feed, and 5% hay and by-products.

Table 4.1 *Comparison between the dataset of the test group (n=996 successful records) and the Dutch dairy herd (data from the Dutch annual report of 2018 of CRV and diet composition from the report "Dierlijke mest en mineralen" of 2017 of CRV). (CRV, 2018; CRV, 2019).*

Parameter	Dutch dairy herd (2018)	Test group (2018-2019)
Breed	>90% HF	79% HF
Parity (%)		
Heifers	32.6	33.0
2nd lactation	27.4	26.5
3rd lactation	18.4	17.8
4th lactation	10.7	14.0
>4th lactation	10.9	8.7
Lactation stage (%) [1]		
1-50 days	14.3	10.5
51-100 days	14.3	18.4
101-150 days	14.3	14.9
151-200 days	14.3	15.3
201-250 days	14.3	15.5
251-300 days	14.3	12.3
301+ days	14.2	13.1
Milk yield (kg FPCM)	29.7	32.4
DMI (kg DM)	20.3	21.5
Diet (%)		
Grass silage	35.2	31.8
Maize silage	18.7	23.7
Fresh grass	13.4	12.1
Compound feed	28.0	25.2
Hay	0.7	1.0
By-products	4.0	5.8

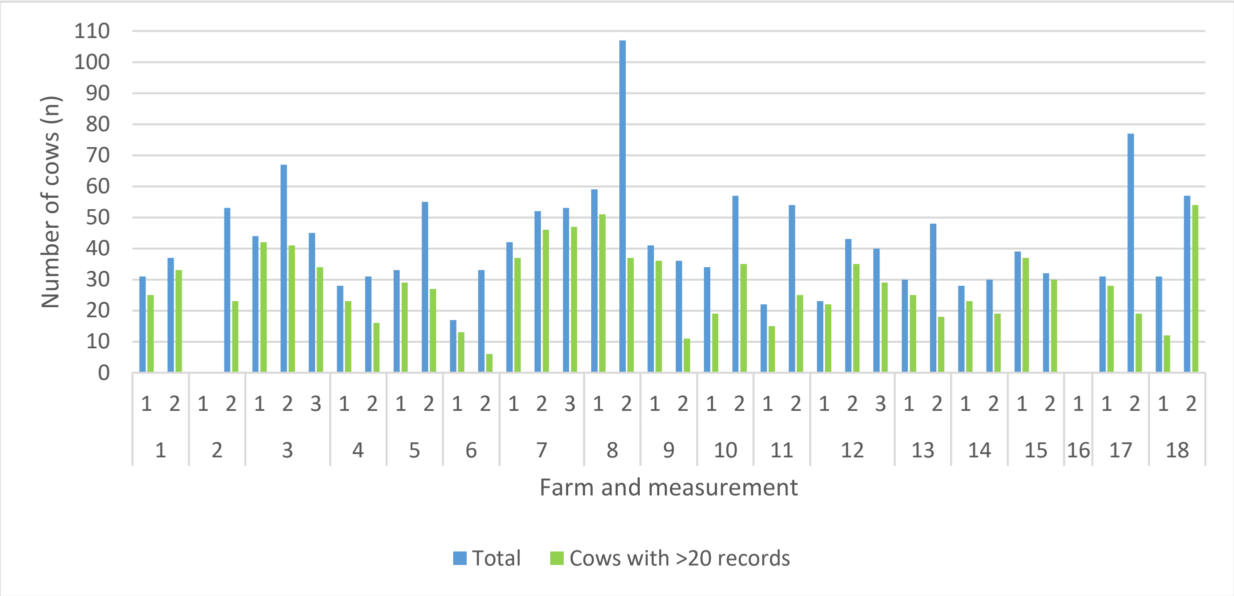
[1] Of the Dutch dairy herd it is known that lactation stage is equally distributed, numbers are estimated on this fact.

[2] The SD shows how measurements are distributed from the average, the average plus and minus one time the SD cover around 68% of all measurements, the average plus and minus two times the SD cover around 95% of all measurements.

4.2 Observed CH_4 emission of the Dutch dairy herd

In total 1718 records of 1278 dairy cows, mostly Holstein-Friesian, from 18 farms were collected between September 10th 2018 and October 14th 2019. Cows with less than 20 valid CH_4 measurements were excluded from the analysis (n=612), resulting in 1106 records of cows with 20 measurements or more. Three farms were measured three times, one farm was measured once and the remaining 14 farms were measured twice. Cows with days in lactation higher than 500 were excluded from the analysis (n=4) as these skew the data and are not representative for the Dutch dairy herd. Parity was grouped per lactation number (1, 2, 3, 4 and 5+). For farm 2 the first measuring period failed to produce cows with 20 records or more and of farm 16 no milk data was available. Due to missing values (either failing cow identification or missing milk samples), 106 units were excluded from the analysis. In total 996 successful records of 791 cows were left for analysis. Figure 4.1 shows the total number of cows measured per farm per measuring period and the number of cows with more than 20 records for both CH_4 and milk production. More detailed information of the measurements per farm are summarized in appendix 2.

Figure 4.1 *Number of cows per farm (1 to 18) per measuring period (1, 2, or 3) measured (both CH_4 and milk production) in total (blue bar) and with more than 20 records (green bar).*



The average CH_4 production (± SD) was 437±94 g CH_4/cow/day (n=996), the average CH_4 intensity was 14.4±5.1 g CH_4/kg FPCM (median is 13.6 g CH_4/kg FPCM, data was not normal distributed). The outcome parameters are shown in table 4.2 and appendix 1.

Table 4.2 *Summary (average, SD, minimum and maximum value) of the dataset (n=996). For the complete summary of all parameters that have been recorded, see appendix 1.*

	Average	SD	Min	Max
CO_2 production (g CO_2/cow/day)	13022	1715	7464	18082
CH_4 production (g CH_4/cow/day)	437	94	186	738
CH_4 intensity (g CH_4/kg FPCM)	14.4	5.1	5.8	70.2
FPCM (kg/cow/day)	32.4	8.6	4.6	62.4
Lactation number	2.4	1.5	1	9
Days in lactation	178	103	11	489
Estimated DMI (kg)	21.5	2.6	11.5	25.8

4.3 Analysis of the variation in enteric CH4

4.3.1 Inventory of the variation observed

The CH_4 intensity was log-transformed in order to meet the assumption of normal distribution. Data was normally distributed after log-transformation (appendix 3). A LMM analysis fitted with REML was performed to estimate which part of the observed total variance was accounted for by the various variance components. The basic model (model 1) containing the variance components between-farm, within-farm, between-cow and within-cow (see chapter 3.5.1) showed a total variance of 4.3 g CH_4/kg FPCM. The variance coefficient (variation expressed as a percentage of the average CH_4 intensity) is 30%. Most variation was observed within cows over measurements, followed by variation within farms over measurements. The model had difficulties estimating the size of each variance component, which is explained in chapter 4.3.2. It resulted in under- or overestimation of variance components when the model is not corrected for fixed effects.

4.3.2 Inventory of the fixed effects

A LMM analysis fitted with REML was performed to indicate which fixed effects might affect the variation of enteric CH_4 intensity within this inventory. The results are summarized in figure 4.2 and appendix 4. In the final model (model 5) most of the variation within farms over measurements is explained, leaving more variation between cows (within a farm) unexplained.

The final model contained the fixed effects soil type, grazing related to season, lactation stage, parity, lactose and urea content of the milk and the lactation value. The variation explained by this model was around 48 to 51%. Comparing the unadjusted partial R^2 of the conditional with the marginal effects revealed that the included fixed effect were additional (table 4.3 and 4.4). Every fixed effect explained an additional part of the observed total variance. The order of adding fixed effects in the model had no substantial effect on the variation explained by each effect.

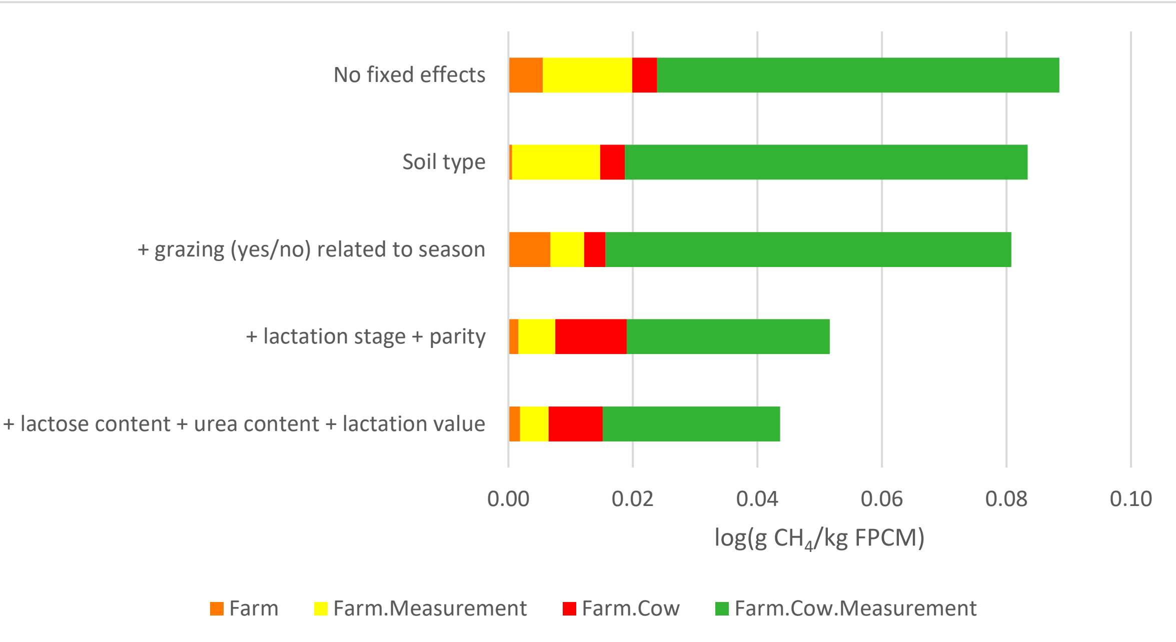


Figure 4.2 *The effects of the fixed variables (soil type, grazing related to season, lactation stage, parity, lactose and urea content in the milk and lactation value) when sequentially added to the model on the variance components (between-farm in orange, within-farm over measurements in yellow, between-cow within a farm in red and within-cow over measurements in green). The figure also shows the reduction of the total variance due to the variation explained by the fixed effects. Note that the scale of the estimates is log-transformed.*

Lactation stage and parity explained most of the variation (around 32%), specifically within-cow variation over measurements. The content of lactose and urea in the milk and the lactation value explained around 8% of the total variation. This was mainly between-cow and within-farm (over measurements) variation. Soil type explained around 6% of the variation which was almost exclusively between-farm variation. Grazing related to season explained 3% of the variation and affected mainly within-farm variation over measurements. The model showed that most variation is on cow level rather than farm level, especially within cows over measurements (*i.e.* over time). In the final model 65% of the total variation was allocated to within-cow variation. Around 20% was allocated to between-cow, 11% to within-farm and only 4% to between-farm variation.

Table 4.3 *Conditional effects including the corresponding unadjusted partial R2 of each model with Y expressed as g CH4/kg FPCM log-transformed.*

Model	Fixed effects	Total variance	Conditional effect	Partial R^2
1	No fixed effects	0.08848	-	-
2	Model 1 + soil type	0.08339	-0.00509	5.75%
3	Model 2 + grazing related to season	0.08079	-0.00260	2.94%
4	Model 3 + lactation stage + parity	0.05161	-0.02918	32.98%
5	Model 4 + lactose + urea + lactation value	0.04367	-0.00794	8.97%

Table 4.4 *Marginal effects including the corresponding unadjusted partial R^2 of each of the (groups of) fixed effect alone with Y expressed as g CH_4/kg FPCM log-transformed.*

Fixed effect	Total variance	Marginal effect	Partial R^2
Soil type	0.08339	-0.00509	5.75%
Grazing related to season	0.08567	-0.00281	3.18%
Lactation stage and parity	0.06056	-0.02792	31.56%
Lactose content, urea content and lactation value	0.08183	-0.00665	7.52%

4.3.3 Inventory of the effect of feed

4.3.3.1 Feed characteristics

Soil type explained most of the variation between farms, but soil type of the location of a farm is not adjustable and does not have a reduction potential. For practical purposes feed strategies to reduce enteric CH_4 (per kg FPCM) are more valuable. Feed characteristics were therefore added to the model to investigate if these effects further explained the variation of CH_4 intensity. Feed information was collected on group level, consequently only the variance components between-farm and within-farm (over measurements) were present. The model (model 6) was corrected for the animal characteristics lactation stage, parity, lactose and urea content in the milk and the lactation value. To select which of the 23 feed characteristics were of interest for the REML analysis, an all subset regression was performed. Percentages of the by-products soy ($p=0.007$) and wheat ($p=0.056$) were most prevalent from the regression. Feed components that were expected to show an effect were added as well: percentage of maize silage, fresh grass, compound feed and the estimated DMI.

In the REML analysis only the percentage of soy as a by-product had a significant effect on the variation on CH_4 intensity (table 4.5). Soil type was added to the model due to the large effect on between-farm variation (see chapter 4.3.2). Soil type together with the percentage of soy showed a significant effect. Around 34% of the between- and within-farm variation was explained. Other selected feed characteristics did not decrease the variance further than soil type. Soil type cannot be replaced with one (or a few) unidimensional (feed) characteristics investigated within this inventory.

Table 4.5 *Analysis output of REML including feed characteristics. Model 6 is corrected lactation stage, parity, lactose and urea content in the milk and the lactation value. Shown are the models with fixed effects that were significant (p<0.05). Note that the data is log transformed.*

Model	Fixed effects	FARM Variance component	s.e.	FARM.MEASUREMENT Variance component	s.e.	Residual variance	Partial R^2
6	Lactation stage + parity + lactose + urea + lactation value	0.0021	0.0033	0.0118	0.0038	0.0139	-
7	Model 6 + Soil type	0.0000	-	0.0112	0.0028	0.0112	19.4%
8	Model 6 + %Soy	0.0000	-	0.0116	0.0028	0.0116	16.5%
9	Model 6 + Soil type + %Soy	0.0000	-	0.0092	0.0023	0.0092	33.8%

4.3.3.2 The effect of fresh grass

Within this inventory the REML analysis showed no effect of the percentage of fresh grass in the feed. However, there were some indications (not statistically substantiated) that fresh grass might reduce the CH_4 production. The average fresh grass intake during grazing (19 measuring periods) was between 0.7% (0.2 kg DM/cow/day) and 61.3% (14.1 kg DM/cow/day. Overall averages (records from the pasture and barn GF together) were not affected by feed characteristics, but when the data of the units were split, it appeared that during grazing the CH_4 production was lower. Of the 9 farms that measured CH_4 with one barn and one pasture GF, 3 farms were emphasized here. These farms had both a sufficient grazing time and sufficient visits in the pasture GF.

In pasture the average CH_4 production was between 230 and 300 g CH_4/cow/day (table 4.6). In the barn the average CH_4 production was between 343 and 367 g CH_4/cow/day. In appendix 6 a visualisation of the data is presented. Wind did not affect the measurements, only the concentration of CH_4 was lowered and not the concentration of CO_2.

Table 4.6 *Summary of the CH_4 production of the barn and pasture records from three measurements during grazing on three different farms. The table also shows the percentage of roughage (grass and maize silage) intake when the cows were in the barn and fresh grass intake when the cows where grazing. The remaining percentage of feed intake where by-products and compound feed.*

Farm	Total emission (g CH_4/cow/day)	In barn (g CH_4/cow/day)	In pasture (g CH_4/cow/day)	Records Barn	Records pasture	%roughage	%fresh grass
4	338	367	300	441	183	45	31
8	304	365	230	1236	533	32	47
13	284	343	246	587	498	36	35

4.4 Evaluation of model calculations to estimate enteric CH_4

The estimated CH_4 intensity was compared with the measured CH_4 intensity on farm level (figure 4.3). A lack of fit analysis indicated a large fitted bias ($F=45.38$, $p<0.001$). The regression model that corresponded with the model versus measured CH_4 intensity was:

$$Calculated\ CH_4\ intensity\ (g\ CH_4/kg\ FPCM) = 9.897 + 0.1694 * Measured\ CH_4\ intensity\ (g\ CH_4/kg\ FPCM)$$

The measured CH_4 intensity is not significantly related to the calculated CH_4 intensity ($p=0.179$). The same analysis was done with CH_4 yield (using estimated DMI) and CH_4 production. Observed results were in agreement with the results for CH_4 intensity. Appendix 7 visualizes the results for the CH_4 yield and CH_4 production. A farm effect was observed, the deviance decreased from 66.88 to 60.15 when adding farm to the model. This indicates that the difference between the CH_4 model calculations and measurements were dependent on farm. For some farms the difference was considerably larger than for other farms.

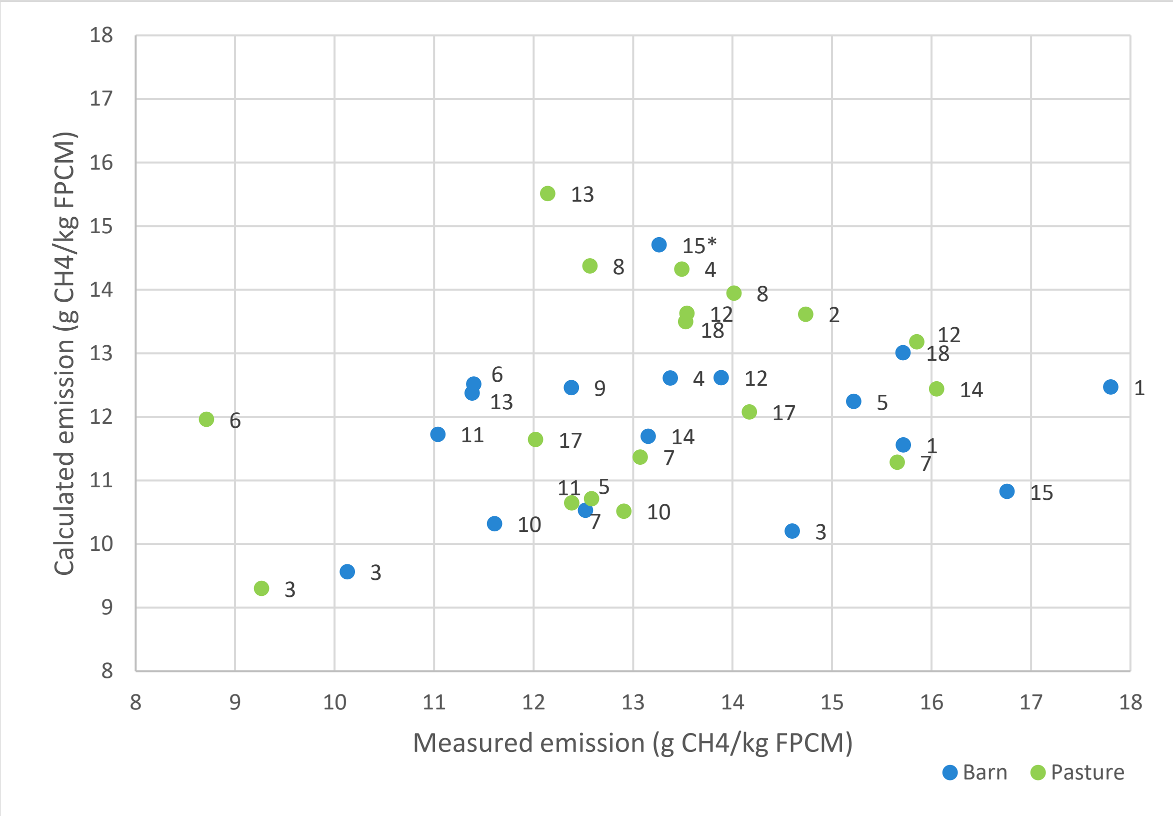


Figure 4.3 *Estimated CH_4 intensity using model calculations plot against the measured CH_4 intensity using the GF. In green the measurements during pasture season and in blue the measurements during barn season. Each dot represents an average per measurement per farm, the corresponding farm number is shown in the graph. Farm 15* is a barn measurement, but with a cut-and-carry system (fresh grass, but zero grazing).*

5 Discussion and conclusions

The first objective of this study was to gain insight in the average CH_4 emission and variation of the Dutch dairy herd, since data of enteric CH_4 on commercial dairy farms within the Netherlands were not available. The test group is representative for the Dutch dairy herd based on milk production, feed intake and diet composition. Therefor the observed average CH_4 production and variation is expected to be representative for the Dutch dairy sector. The representativity of this study is confirmed by a comparison of the observed emission with the emission according to the Dutch National Inventory. The average measured CH_4 production from September 2018 to October 2019 was 437±94 g CH_4/cow/day and the CH_4 yield was 14.4±5.1 g CH_4/kg FPCM. This was based on an estimated feed intake of 21.5 kg DM/day and a milk yield of 32.4 kg FPCM/day. The results are in agreement with the estimated average CH_4 emission of the Dutch dairy sector based on the IPCC Tier 3 approach in 2017; CH_4 production of 401 g CH_4/cow/day and CH_4 intensity of 13.5 g CH_4/kg FPCM (Ruyssenaars *et al.,* 2019; Van Bruggen *et al.,* 2019). This was based on a daily DMI of 20.8 kg and daily milk yield of 29.7 kg FPCM (Van Bruggen *et al.,* 2019). The reference diet used in the National Inventory Report consisted of 11% grass herbage, 38% grass silage, 21% maize silage, 25% compound feed and 5% wet by-products (Ruyssenaars *et al.,* 2019). Within this inventory, the average diet consisted of 12% fresh grass, 32% grass silage, 24% maize silage, 25% compound feed and 7% by-products. Thus, overall the feed composition, DMI and milk yield of this study is comparable to the National Inventory, as well as the observed enteric CH_4 emission. The variation observed is also comparable with the variation seen in experiments, which is between animals on the same *ad libitum* diet around 60 g CH_4/cow/day or, expressed as a percentage of the average (variation coefficient), around 18% (e.g. Grainger *et al.,* 2007). The variation found in this inventory is somewhat higher (94 g CH_4/cow/day, 21.5%) due to farm to farm differences in diets, feeding strategies, breeds and farm management.

The second objective of this study was to investigate which farm and cow characteristics potentially influenced the variation in enteric CH_4. Approximately 49% of the total observed variation is explained by farm and cow characteristics. These effects included the dominant soil type of the farm (sand, clay, peat), whether or not cows were grazing related to the time of the year, days in lactation, parity, the content of urea and lactose of the milk and the lactation value. Days in lactation and parity explained most of the variation (around 32%). The impact of parity on the CH_4 intensity was expected, since heifers have a higher CH_4 intensity compared to older lactating cows. Heifers are growing animals that use part of the energy intake for growing at the expense of milk production. The impact of days in lactation was also expected. Early lactating cows are in a negative energy balance and thus part of the milk production is based on energy from body reserves resulting in a lower CH_4 intensity. Late (and mid) lactating cows produce less milk and use part of their energy (feed) for building up fat reserves and growth of the foetus. Additionally, early lactating cows generally receive a diet with a higher percentage of compound feed and a lower percentage roughages. This diet, combined with a higher feed intake, reduces CH_4 yield.

Most variation was seen on cow level (between cows within a farm and within cows over measurements) rather than farm level (between farms and within farms over measurements). Feed intake, feed composition and the production level of cows change during the year. Production of CH_4 is strongly related to this pattern. There is still a substantial part of variation between cows (20%) that is not explained by the variables mentioned before. This could indicate that there is room for further reductions of CH_4 emission. Other cow characteristics, like the composition and functioning of the rumen microbiome or genetics, are worthwhile investigating.

Soil type reduced the between-farm variation to almost completely. Feed composition was expected to explain this, since soil type is related to what farmers grow on their land. Farms in regions dominated by sandy soil tend to grow more maize silage compared to clay dominated regions. Feed data analysis did not support this hypothesis. No effect of the percentage of maize silage nor other feed characteristics was found on CH_4 intensity, except the percentage of soy as a by-product together with soil type. Replacing grass silage by maize silage is expected to result in a reduction of CH_4 yield up to 5% due to the a higher starch and lower fibre content (Van Gastelen *et al.,* 2019a). For CH_4 intensity this effect was not found within this inventory by comparing farms with high and low maize silage contents in the diet. Respiration chamber studies show that a substantial part (up to 100%) of the grass silage needs to be replaced by maize silage in order to have an effect (Van Gastelen *et al.,* 2014; Van Gastelen *et al.,* 2019a). None of the farms within this inventory used such a feeding strategy (maximum of maize silage in the roughage was 70%). Combined with other feed factors that influence CH_4 production, like adjusting the compound feed based on the roughage composition, no effect of maize silage on CH_4 intensity was found. Other explanations for the observed differences might be the quality components of the roughage since different types of soil grow different quality of feeds (Hoving & Velthof, 2006). None of the quality components that were collected in this inventory showed an effect in the model. It is important to note that not all data of the quality components were available. No single unidimensional feed component was directly correlated to a higher or lower CH_4 intensity. The percentage of soy as a by-product did show an effect, but not as much as soil type did and interpretation of this effect is difficult. Soy as a by-product might not have been the only soy in the diet, compound feed might also contain soy which was not taken into account. Statistical analysis was also performed using the uncorrected model (model 1), no different conclusion was found (appendix 5). These findings suggest that soil type is not directly exchangeable for one (feed) characteristic investigated within this inventory. An unknown factor confounded with soil type could be an explanation for this result. It raises the question whether or not soil type specific measurements are needed to effectively reduce the average Dutch enteric CH_4 emission.

Comparing measurements of the pasture Greenfeed with barn Greenfeed indicated that grazing might result in a lower CH_4 production, this was not statistically substantiated. Not much research has been done on the effect of grazing on CH_4 emission. Some research shows a reducing potential of both partial grazing and cut-and-carry on both CH_4 yield and CH_4 intensity. Cameron *et al.* (2018) found reductions of CH_4 production of 17% (cut-and-carry) and 39% (partial grazing) when around 33% of the total mixed ration diet was replaced with fresh grass. The percentage of fresh grass in the diet did not explain any of the observed variation in enteric CH_4 intensity in this inventory. However, grazing related to season explained a small part of the variation in CH_4 intensity (3%). The small effects and indications can be explained by multiple factors. The amount of records collected on the pasture (1-30% of the total) was low and might have been too few to detect any effects. The effect of fresh grass on CH_4 intensity might also not be visible due to the substantial amount of supplemental feed fed in the barn. At last, fresh grass quality was not taken into account within this inventory. Grass quality varies largely over the year, between pastures and even within a day. A controlled experimental study of the effect of fresh grass on the reduction of CH_4 is needed to draw any conclusions.

A model to calculate the enteric CH_4 emission based on the diet fed, feed quality and (estimated) feed intake was evaluated for use in practice. A comparison of the average herd emission of single farms revealed no correlations between the CH_4 emission measured in this inventory and the CH_4 emission as estimated with the model. This suggests that the model can only accurately estimate the CH_4 emission within a limited range around the average Dutch emission. In practice, a large variation in feeding strategies is observed which is not incorporated into the model. These results implicate the necessity to further develop the model before practical implementation. For future validation of the model using the Greenfeed system, the background emission of farms should be taken into account. The background emission should be relatively stable in order for the Greenfeed to accurately measure CH_4 production of dairy cows. On commercial dairy farms the background emission is not always stable. A relatively high number of animals (compared to research facilities), different flooring and ventilation can cause unstable background emissions. This results in a higher inaccuracy of the measured CH_4 production. The effect of farm found in the comparison can be both caused by an unstable background and unique feeding strategies which exceed the range of the model. For example, on one farm the measured CH_4 emission was consequently higher than the model calculations. This specific farmer applied a unique strategy for growing and harvesting grass. Measurements that were consequently overestimated by model calculations compared to measured estimations were often measurements during grazing in spring. Follow-up research focusing on feeding strategies used by farms that showed more inaccuracy between model calculations and measured emissions will most likely result in a more robust model for practical application.

References

Arthur. P.F., Barchia, I.M., Weber, C., Bird-Gardiner, T., Donoghue, K.A., Herd, R.M., & Hegarty, R.S. (2017). Optimizing test procedures for estimating daily methane and carbon dioxide emissions in cattle using short-term breath measures. *Journal of Animal Science, 95*(2), 645–656. https://doi.org/10.2527/jas.2016.0700

Bannink, A., Van Schijndel, M.W., & Dijkstra, J. (2011). A model of enteric fermentation in dairy cows to estimate methane emission for the Dutch National Inventory Report using the IPCC Tier 3 approach. *Animal Feed Science and Technology*, *166*, 603-618.

Cameron, L., Chagunda, M.G.G., Roberts, D.J., & Lee, M.A. (2018). A comparison of milk yields and methane production from three contrasting high-yielding dairy cattle feeding regimes: Cut-and-carry, partial grazing and total mixed ration. *Grass and Forage Science*, *73*(3), 789-797.

CBS, PBL, RIVM, & WUR (2017). Greenhouse gas emissions, 1990-2016 (indicator 0165, version 31, 6 September 2017). www.environmentaldata.nl. Statistics Netherlands (CBS), The Hague; PBL Netherlands Environmental Assessment Agency, The Hague; RIVM National Institute for Public Health and the Environment, Bilthoven; and Wageningen University and Research, Wageningen.

Coenen, P.W.H.G., Van der Maas, C.W.M., Zijlema, P.J., Arets, E.J.M.M., Baas, K., van den Berghe, A.C.W.M., Van Huis, E.P., Geilenkirchen, G., Hoogsteen, M., Spijker, J., Te Molder, R., Dröge, R., Montfoort, J.A., Peek, C.J., Vonk, J., Oude Voshaar, S., & Dellaert, S. (2017). Greenhouse gas emissions in the Netherlands 1990-2015: National Inventory Report 2017. Bilthoven, RIVM National Institute for Public Health and the Environment, RIVM Report 2017-0033.

CRV. (2009). CRV Jaarstatistieken 2008 Nederland. Arnhem, CRV BV. https://crvnl-be6.kxcdn.com/wp-content/uploads/2014/08/Jaarstatistieken-CRV-2008.pdf

CRV. (2018). Dierlijke mest en mineralen 2017. Den Haag, Centraal Bureau voor de Statistiek.

CRV. (2019). CRV Jaarstatistieken 2018, voor Nederland. Arnhem, Coöperatie CRV.

Dijkstra, J., Bannink, A., France, J., Kebreab, E., & Van Gastelen, S. (2018). Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. *Journal of dairy science*, *101*(10), 9041-9047.

Grainger, C., Clarke, T., McGinn, S. M., Auldist, M. J., Beauchemin, K. A., Hannah, M. C., Waghorn, G.C., Clark, H., & Eckard, R. J. (2007). Methane emissions from dairy cows measured using the sulfur hexafluoride (SF6) tracerf and chamber techniques. *Journal of dairy science*, *90*(6), 2755-2766.

Hoving, I.E., Velthof, G.L. (2006). Landbouw- en milieukundige effecten van graslandvernieuwing op zand- en kleigrond, Lelystad, Animal Sciences Group Wageningen UR, PraktijkRapport Rundvee 83.

Huhtanen, P., Cabezas-Garcia, E. H., Utsumi, S., & Zimmerman, S. (2015). Comparison of methods to determine methane emissions from dairy cows in farm conditions. *Journal of dairy science*, *98*(5), 3394-3409.

Lagerwerf, L.A., Bannink, A., Van Bruggen, C., Groenestein, C.M., Huijsmans, J.F.M., Van der Kolk, J.W.H., Luesink, H.H., Van der Sluis, S.M., Velthof, G.L., & Vonk, J. (2019). Methodology for estimating emissions from agriculture in the Netherlands. Calculations of CH4, NH3, N2O, NOx, NMVOC, PM10, PM2.5 and CO2 with the National Emission Model for Agriculture (NEMA) – update 2019. Wageningen, The Statutory Research Tasks Unit for Nature and the Environment. WOt-technical report 148.

Lovett, D.K., Shalloo, L., Dillon, P., & O'Mara, F.P. (2006). A systems approach to quantify greenhouse gas fluxes from pastoral dairy production as affected by management regime. *Agricultural Systems*, *88*(2-3), 156-179.

Manafiazar, G., Zimmerman, S., & Basarab, J. A. (2016). Repeatability and variability of short-term spot measurement of methane and carbon dioxide emissions from beef cattle using GF emissions monitoring system. *Canadian Journal of Animal Science*, *97*(1), 118-126.

McAllister, T. A., & Newbold, C. J. (2008). Redirecting rumen fermentation to reduce methanogenesis. *Australian Journal of Experimental Agriculture, 48*(2), 7-13.

Ruyssenaars, P.G., Coenen, P.W.H.G., Zijlema, P.J., Arets, E.J.M.M., Baas, K., Dröge, R., Geilenkirchen, G., ’t Hoen, M., Honig, E., Van Huet, B., Van Huis, E.P., Koch, W.W.R., Lagerwerf, L.A.., Te Molder, R., Montfoort, J.A., Peek, C.J., Vonk, J., Van Zanten, M.C. (2019). Greenhouse gas emissions in the Netherlands 1990–2017: National Inventory Report 2019. Bilthoven, RIVM National Institute for Public Health and the Environment, RIVM Report 2019-0020

Šebek, L.B., de Haan, M.H.A., Bannink, A. (2014). Methaanemissie op het melkveebedrijf Impactanalyse voor reductiemaatregelen en doorrekening daarvan in de Kringloopwijzer. Wageningen, Wageningen UR (University & Research centre) Livestock Research, Livestock Research Rapport 796.

Šebek, L.B., Mosquera, J., Bannink, A. (2016). Rekenregels voor de enterische methaanemissie op het melkveebedrijf en reductie van de methaanemissie via mest-handling; het handelingsperspectief van het voerspoor inzichtelijk maken met de Kringloopwijzer. Lelystad, Wageningen UR (University & Research centre) Livestock Research, Livestock Research Rapport 976.

Van Bruggen, C., A. Bannink, C.M. Groenestein, J.F.M. Huijsmans, L.A. Lagerwerf, H.H. Luesink, S.M. van der Sluis, G.L. Velthof & J. Vonk (2019). Emissies naar lucht uit de landbouw in 2017. Berekeningen met het model NEMA. Wageningen, WOT Natuur & Milieu, WOt-technical report 147.

Van Gastelen, S., Antunes Fernandes, E. C., Hettinga, K. A., Klop, G., Alferink, S. J. J., & Dijkstra, J. (2014). *Replacing grass silage with maize silage affects rumen fermentation characteristics and enteric methane production in dairy cattle.* Abstract from 39th Animal Nutrition Research (ANR) Forum, Utrecht, The Netherlands.

Van Gastelen, S., Bannink, A., & Dijkstra, J. (2019a). Effect of silage characteristics on enteric methane emission from ruminants. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *14*(51), 051.

Van Gastelen, S., Dijkstra, J., & Bannink, A. (2019b). Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep?. *Journal of dairy science, 102*(7), 6109-6130.

Velazco, J. I., Herd, R. M., Cottle, D. J., & Hegarty, R. S. (2017). Daily methane emissions and emission intensity of grazing beef cattle genetically divergent for residual feed intake. *Animal Production Science, 57*(4), 627-635.

Zom, R. L. G. (2014). The development of a model for the prediction of feed intake and energy partitioning in dairy cows (Doctoral dissertation, Wageningen University).

Appendix 1 Parameter outcomes

Appendix 1.1 *Average, SD, minimum and maximum of collected parameters. Data is from the cows with 20 records or more (n=996). Feed data is calculated on group level (n=37), except for the estimated feed intake (in kg DMI) which is shown on individual level.*

Variable	Average	SD	Min	Max
Methane data				
CO_2 production (g CO_2/cow/day)	13021.5	1714.8	7463.6	18082.3
CH_4 production (g CH_4/cow/day)	436.7	94.1	185.9	738.0
CH_4 intensity (g CH_4/kg FPCM)	14.4	5.1	5.8	70.2
Number of Records per cow	40.7	18.8	20	188
Milk data				
FPCM (kg/day)	32.4	8.6	4.6	62.4
Milk yield (kg/day)	30.6	9.4	3.9	60.3
Expected milk yield (kg/day)	30.3	8.7	5.6	58.2
Individual standard cow (ISK)	47.0	10.6	0.4	77.6
Fat (%)	4.48	0.80	2.37	8.84
Protein (%)	3.68	0.43	2.61	7.16
Lactose (%)	4.54	0.18	3.76	5.21
Urea (mg/100 g)	20.4	6.12	5	63
Kg Fat Protein (kg)	2.44	0.62	0.40	4.67
Lactation number	2.4	1.5	1	9
Days in lactation	178.0	102.6	11	489
Lactation value	102.8	12.0	46	150
Feed data				
Estimated feed intake (kg DMI)	21.5	2.6	11.5	25.8
Grass silage (kg and % DM)	6.8 (31.8%)	2.8 (14.8%)	1.2 (5.6%)	13.8 (70.0%)
Maize silage (kg and % DM)	5.3 (23.7%)	2.8 (12.3%)	0.0 (0.0%)	11.3 (47.0%)
Fresh grass (kg and % DM)	2.6 (12.1%)	3.6 (16.3%)	0.0 (0.0%)	14.2 (61.3%)
Compound feed (kg and % DM)	5.5 (25.2%)	1.7 (7.6%)	0.4 (1.9%)	8.3 (35.4%)
Hay products (kg and % DM)	0.2 (1.0%)	0.7 (3.1%)	0.0 (0.0%)	3.6 (17.0%)
By-products (kg and % DM)	1.3 (5.8%)	2.3 (9.9%)	0.0 (0.0%)	11.0 (48.8%)
- Wheat by-products (%)	1.7	2.9	0.0	9.9
- Maize by-products (%)	2.2	4.0	0.0	15.9
- Soy (%)	0.9	1.5	0.0	5.8
- Brewers grains (%)	0.6	1.4	0.0	6.2
- Potato products (%)	0.1	0.4	0.0	1.9
- Beet products (%)	1.8	2.7	0.0	11.3
- Turnip products (%)	0.3	0.7	0.0	2.7
Neutral detergent fibre (g NDF) (n=33)	5299.5	1426.3	1944.1	7978.2
Starch (g) (n=28)	2107.4	733.9	853.5	3753.1
Feed unit of lactation (VEM)	21750.9	2230.3	13867.5	24998.0
Nitrogen (g N) (n=29)	576.2	46.0	486.5	685.0
Phosphorus (g P) (n=32)	75.5	9.0	60.3	92.5
Crude protein (g RE)	3438.4	349.4	2567.6	4244.9
Digestible crude protein (g VRE) (n=32)	2553.7	326.9	1986.0	3424.0
Digestible true protein (g DVE) (n=36)	1724.1	286.0	328.6	2075.0
Degraded protein balance (g OEB) (n=36)	538.2	343.7	15.4	2270.0

Appendix 2 Farm and measurement information

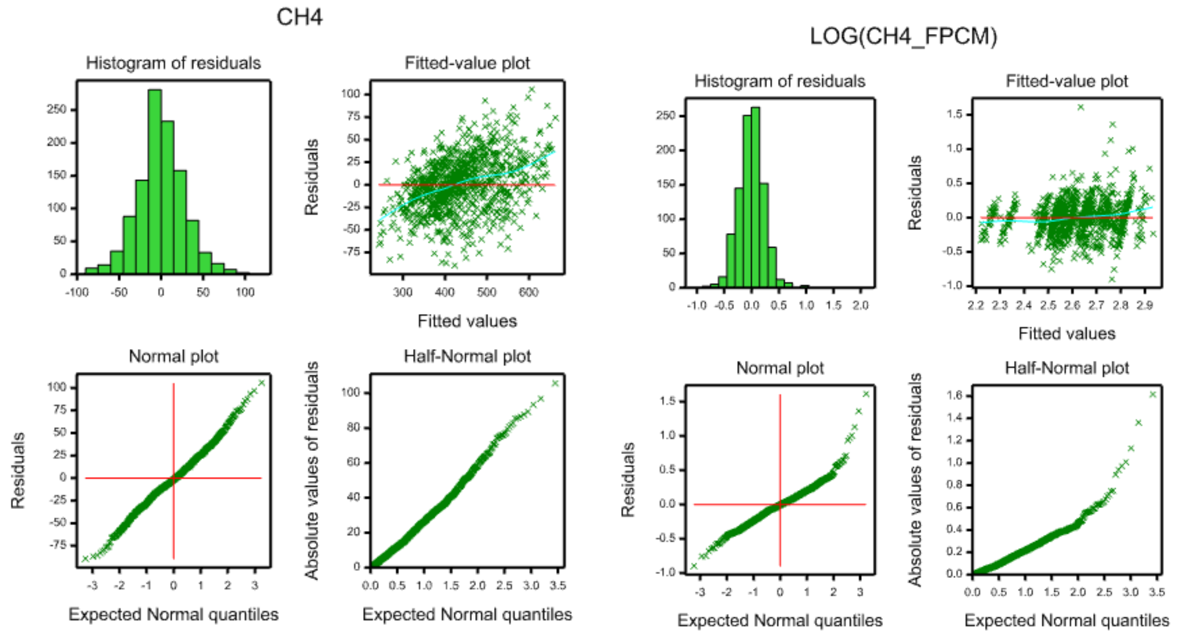
Appendix 2.1 *Information per farm per measurement. From left to right: farm number, measurement number, soil type of the farm, whether or not the cows were grazing during the measurement, number of cows of the complete lactating herd, number of cows of the group that had access to the Greenfeed, number of cows used for the analysis (i.e. with 20 methane records or more and of which milk data was available), average CH_4 production, CH_4 intensity, fat-protein corrected milk (FPCM), parity (lactation number), days in lactation, estimated dry matter intake (DMI), the percentage of grass silage, maize silage, fresh grass, compound feed and by-products the herd received. The last column contains extra notes, like whether or not the farmer had an automatic milking system (AMS). Of two farms (farms 2 and 16) no milk data were available from the first measuring period.*

Farm	Measure-ment	Soil type	Grazing	N herd	N group	N test group	CH_4 production (g CH_4/cow/day) ±SD	CH_4 intensity (g CH_4/kg FPCM) ±SD	FPCM ±SD	Parity ±SD	Days in lactation ±SD	kg DMI [1]	% grass silage	% maize silage	% fresh grass	% compound feed	% by-products	Notes
1	1	Clay	No	111	34	25	590.3±70.8	19.4±6	33.2±9.8	2.2±1.2	192±123	21.8	43.6	20.9	0.0	27.7	7.8	AMS
1	2	Clay	No	113	39	33	579.9±58	17.6±8.1	36.9±10.4	2.4±1	191±106	23.4	43.0	20.4	0.0	28.8	7.8	AMS
2	1	Clay	No	91	13	0							30.3	29.5	0.0	3.3	36.9	
2	2	Clay	Yes	94	53	23	441.5±61.6	15.2±3.3	30±5.1	1.7±0.9	165±96	21.5	24.7	23.6	1.0	1.9	48.8	
3	1	Sand	No	66	45	42	505.8±90.9	14.8±2.9	34.6±5.4	2.9±1.8	109±94	20.3	34.5	39.2	0.0	26.3	0.0	AMS
3	2	Sand	No	77	68	41	433.4±63.2	10.3±1.9	42.8±7.2	3.4±1.4	124±71	24.2	36.6	30.1	0.0	25.4	7.9	AMS
3	3	Sand	Yes	80	46	34	375.3±66.3	9.4±1.8	40.5±6.7	3.3±1.4	130±66	21.7	19.2	30.9	23.6	24.2	2.1	AMS
4	1	Peat	No	105	28	23	438.6±85.7	13.6±2.4	32.8±6.6	3.1±2.3	170±97	21.7	46.2	20.6	0.0	31.2	2.0	
4	2	Peat	Yes	124	32	16	364.9±62.1	16±9.7	27±9.9	2.6±1.4	212±151	21.4	26.6	17.9	31.1	24.5	0.0	
5	1	Clay	No	165	36	29	456±48.7	15.8±3.1	30±6.6	2.4±1	189±86	21.7	42.8	27.2	0.0	24.9	5.1	
5	2	Clay	Yes	184	57	27	437.7±75.5	12.8±2.2	34.8±6.7	2±1.2	153±97	22.1	41.8	27.2	0.7	24.5	5.8	
6	1	Peat	No	105	17	13	348.2±39.4	11.8±2.1	30.5±6.7	3.2±1.6	141±72	20.3	51.3	19.8	0.0	25.8	3.1	
6	2	Peat	Yes	100	34	6	284.9±31.4	8.9±1.8	32.7±4.1	2.7±1.2	113±74	21.4	16.4	18.8	41.6	21.3	1.9	
7	1	Sand	Yes	93	43	37	553.9±78.5	16.4±4.5	35.4±7.7	2.4±1.4	148±113	22.5	26.9	31.0	7.5	34.7	0.0	AMS
7	2	Sand	No	104	60	46	500.4±86.7	12.8±2.8	40±7.7	2.4±1.5	146±88	23.4	32.1	28.5	0.0	35.3	4.2	AMS
7	3	Sand	Yes	109	56	47	466±70.1	13.7±3.9	35.6±7.9	2.5±1.5	218±98	23.0	26.7	26.7	16.8	29.9	0.0	AMS
8	1	Clay	Yes	160	59	51	404.8±54	14.5±3.4	28.9±5.7	1.7±1	142±83	21.7	29.6	21.5	10.4	23.6	14.9	
8	2	Clay	Yes	166	110	37	340.9±77.8	14.6±10.4	27.1±7.2	1.7±1	186±118	21.3	5.6	25.9	46.8	15.3	6.4	
9	1	Sand	No	65	43	36	408±58.3	12.5±1.8	33±4.3	2.5±1.1	157±60	22.4	40.0	26.8	0.0	28.0	5.2	
9	2	Sand	Yes	64	42	12	387.9±71	14.9±5.9	29±9.3	2.6±1.5	200±143							

Farm	Measure-ment	Soil type	Grazing	N herd	N group	N test group	CH_4 production (g CH_4/cow/day) ±SD	CH_4 intensity (g CH_4/kg FPCM) ±SD	FPCM ±SD	Parity ±SD	Days in lactation ±SD	kg DMI [1]	% grass silage	% maize silage	% fresh grass	% compound feed	% by-products	Notes
10	1	Sand	No	94	35	19	437.4±63	11.7±1.6	37.7±7	1.8±1	124±73	22.9	16.8	39.3	0.0	25.2	18.8	AMS
10	2	Sand	Yes	95	61	35	409.2±51.7	13.7±3.8	31.7±7.3	2±1.2	243±97	23.0	14.2	43.6	11.1	26.9	4.0	AMS
11	1	Sand	No	116	24	15	326.7±53.1	11.6±2.6	29.6±8.7	1.6±0.8	217±100	18.5	49.8	23.4	0.0	26.8	0.0	
11	2	Sand	Yes	120	59	25	426.2±81.9	12.8±3.2	34.4±7.3	1.8±1	152±103	20.6	30.4	28.2	15.4	25.0	1.1	
12	1	Clay	Yes	174	27	22	486±77.6	16.2±3.7	30.7±7.5	1.9±1.2	223±112	20.8	38.0	0.0	26.6	35.4	0.0	AMS, organic farmer
12	2	Clay	No	176	45	35	418.6±71.9	14.6±3.5	30.1±8.3	2.1±1.5	240±109	20.7	64.9	0.0	0.0	35.1	0.0	AMS, organic farmer
12	3	Clay	Yes	172	44	29	398.3±45	16.5±10.9	29.4±11.3	2.3±1.8	161±125	21.6	10.2	0.0	61.3	28.5	0.0	AMS, organic farmer
13	1	Sand	No	94	30	25	368.5±45.3	12.2±3.4	32.4±8.8	2.6±1.4	186±95	21.2	44.8	17.0	0.0	34.9	3.3	
13	2	Sand	Yes	90	56	18	318.3±46.4	12.7±2.7	26.2±6.5	2.8±1.6	262±59	20.8	19.4	16.7	34.7	24.4	4.8	
14	1	Clay	No	100	29	23	432.3±49.5	13.4±2.1	32.9±5.9	2.4±1.3	164±59	23.9	32.9	47.0	0.0	20.2	0.0	AMS
14	2	Clay	Yes	92	30	19	455±63	17.2±5.3	28.3±7.7	2.3±1.2	254±120	22.4	17.0	41.0	16.0	24.4	1.6	AMS
15	1	Clay	No	142	39	37	541.4±76.5	17.3±4	32.3±6.7	2.7±1.8	180±101	21.7	32.8	40.6	0.0	26.6	0.0	
15	2	Clay	No	133	32	30	390.5±58.8	13.6±2.8	29.4±5.4	2.9±1.7	173±89	22.1	6.3	36.1	39.6	17.9	0.0	Cut and carry system (zero grazing)
16	1	Löss	No	211	32	0	412.8±39.1			1.6±0.7	375±118	22.6	36.5	25.7	0.0	27.3	10.5	AMS
17	1	Clay	Yes	101	37	28	422.9±76.2	14.5±3.1	29.8±5.6	2.2±1.1	179±114	20.3	39.1	17.9	16.3	19.5	7.2	
17	2	Clay	Yes	102	89	19	403±97	12.8±4.1	33.5±8.2	2.6±1.4	157±116	20.5	19.4	13.4	30.5	28.5	8.2	
18	1	Peat	Yes	38	38	12	344.3±47.6	13.9±2.6	25.4±5.8	2.8±1.2	202±53	17.2	55.9	0.0	18.5	25.6	0.0	
18	2	Peat	No	124	96	54	361.3±63.9	17.7±5.9	23±8.5	3.1±1.5	246±118	15.1	70.0	0.0	0.0	30.0	0.0	AMS

1 Feed intake is an estimated feed intake in kg DMI of the test group

Appendix 3 Normal distribution check for REML



Appendix 3.1 *Histogram and plots of the residuals of the data (CH_4 production on the left and CH_4 intensity log-transformed on the right). CH_4 intensity (g CH_4/kg FPCM) was log-transformed in order to meet the assumption of normal distribution.*

Appendix 4 REML analysis outcome

Appendix 4.1 *Results of the REML analysis where fixed effects are sequentially added to the model with Y expressed as CH_4 intensity (g CH_4/kg FPCM log-transformed).*

Model	Fixed effects	FARM Variance component	FARM s.e.	FARM.MEASUREMENT Variance component	FARM.MEASUREMENT s.e.	FARM.COW Variance component	FARM.COW s.e.	FARM.MEASUREMENT.COW Variance component	FARM.MEASUREMENT.COW s.e.	Residual variance
1	No fixed effects	0.00553	0.00539	0.01435	0.00545	0.00400	0.00425	0.0646	0.00506	0.08848
2	Model 1 + Soil type [1]	0.00059	0.00384	0.01413	0.00531	0.00397	0.00424	0.0647	0.00506	0.08339
3	Model 2 + grazing (yes/no) related to season [2]	0.00675	0.00494	0.00542	0.00311	0.00342	0.00422	0.0652	0.00508	0.08079
4	Model 3 + lactation stage [2] + parity [2]	0.00162	0.00278	0.00590	0.00280	0.01149	0.00284	0.0326	0.00286	0.05161
5	Model 4 + lactose content [1] + urea content [2] + lactation value [2]	0.00188	0.00235	0.00459	0.00222	0.00870	0.00249	0.0285	0.00254	0.04367

1 $p<0.05$ in final model

2 $p<0.001$ in final model

Appendix 5 Effect of feed additional analysis

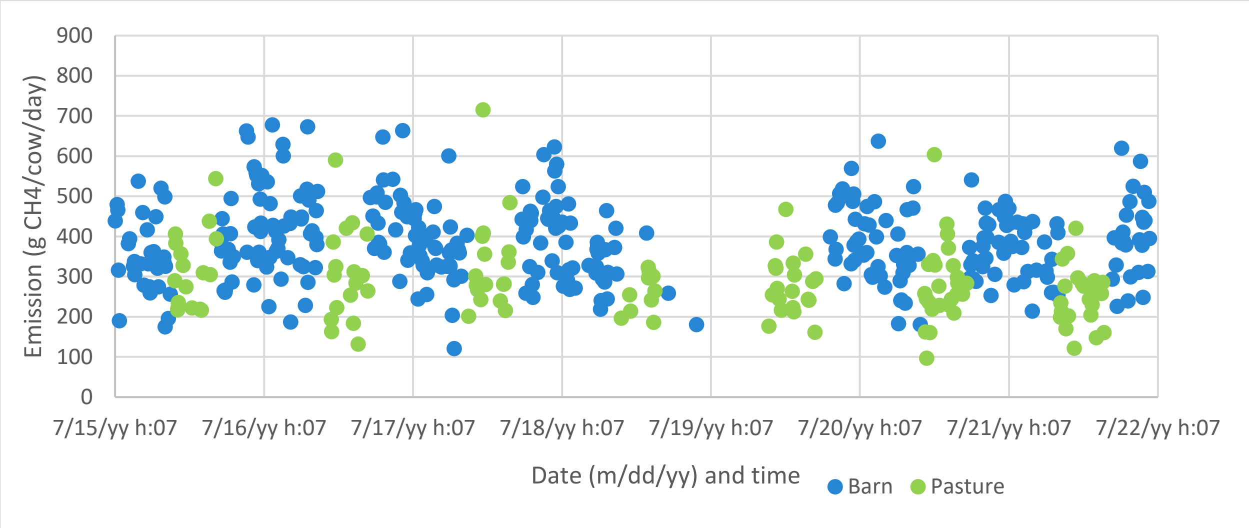
Appendix 5.1 *Analysis output of REML on the between- and within-farm variation for the uncorrected model including feed characteristics. Shown are the models with fixed effects that were significant. Note that the data is log transformed. DMI showed a trend when the data was not corrected for lactation stage, but it did not decrease the total residual variance.*

Model	Fixed effects	FARM Variance component	s.e.	FARM.MEASUREMENT Variance component	s.e.	Residual variance
1	No fixed effects	0.0052	0.0039	0.0011	0.0035	0.0063
2	Model 1 + soil type [2]	0.0013	0.0028	0.0011	0.0035	0.0024
10	Model 1 + %soy [1] + DMI [1]	0.0042	0.0039	0.0107	0.0035	0.0149
11	Model 1 + soil type [2] + %Soy [1]	0.0000	-	0.0116	0.0029	0.0116

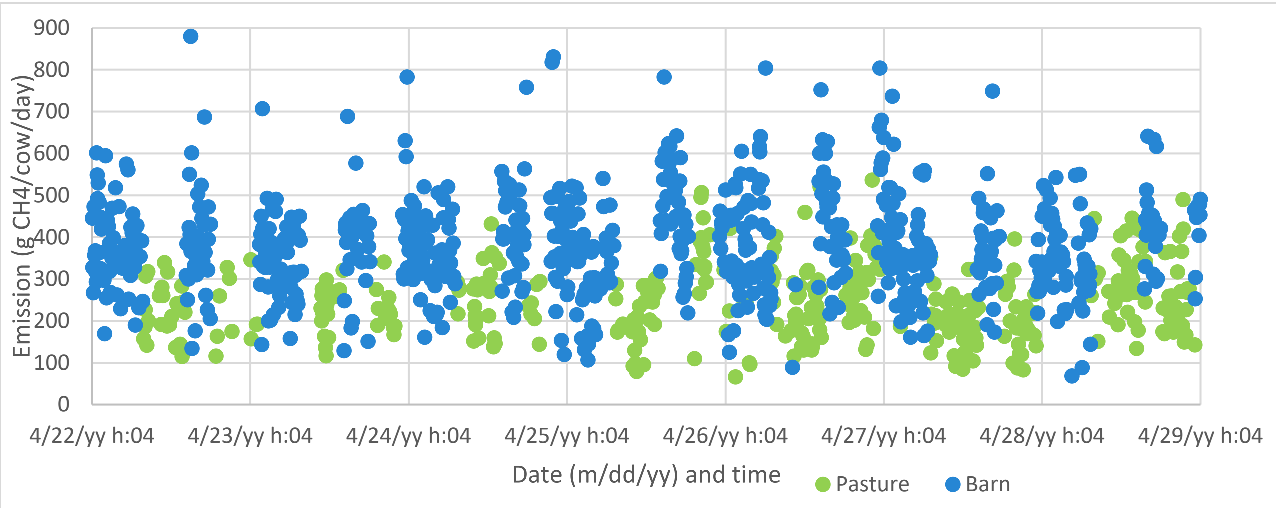
[1] trend 0.05<p<0.1

[2] p<0.05

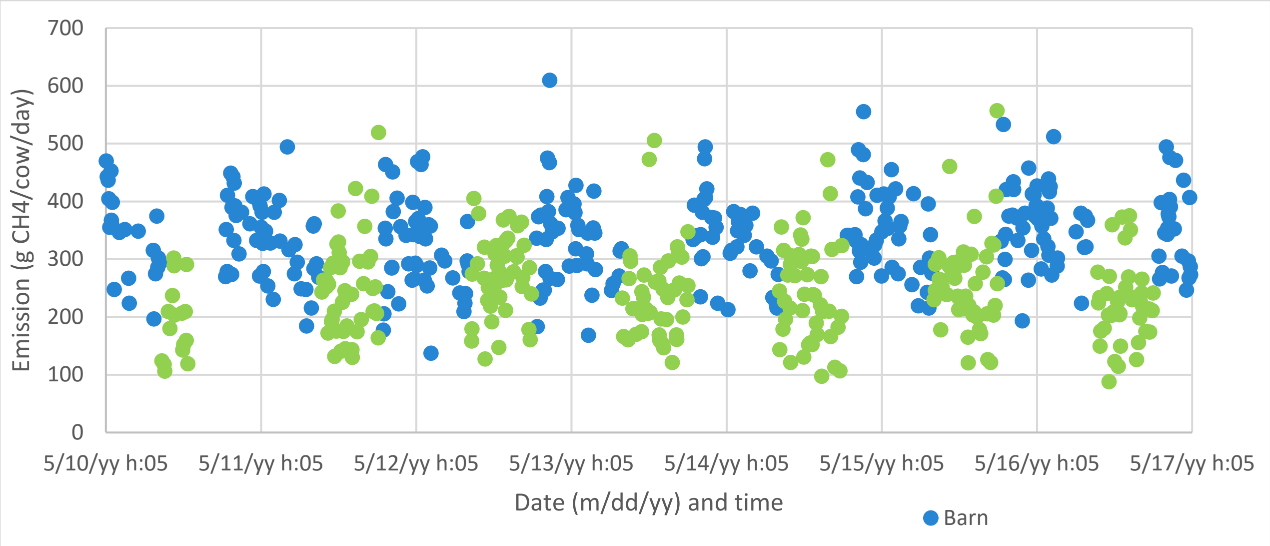
Appendix 6 Barn and pasture records of three selected farms



Appendix 6.1 *Barn and pasture records of all cows from farm 4 in one week of the two week measuring period in July 2019 during grazing.*

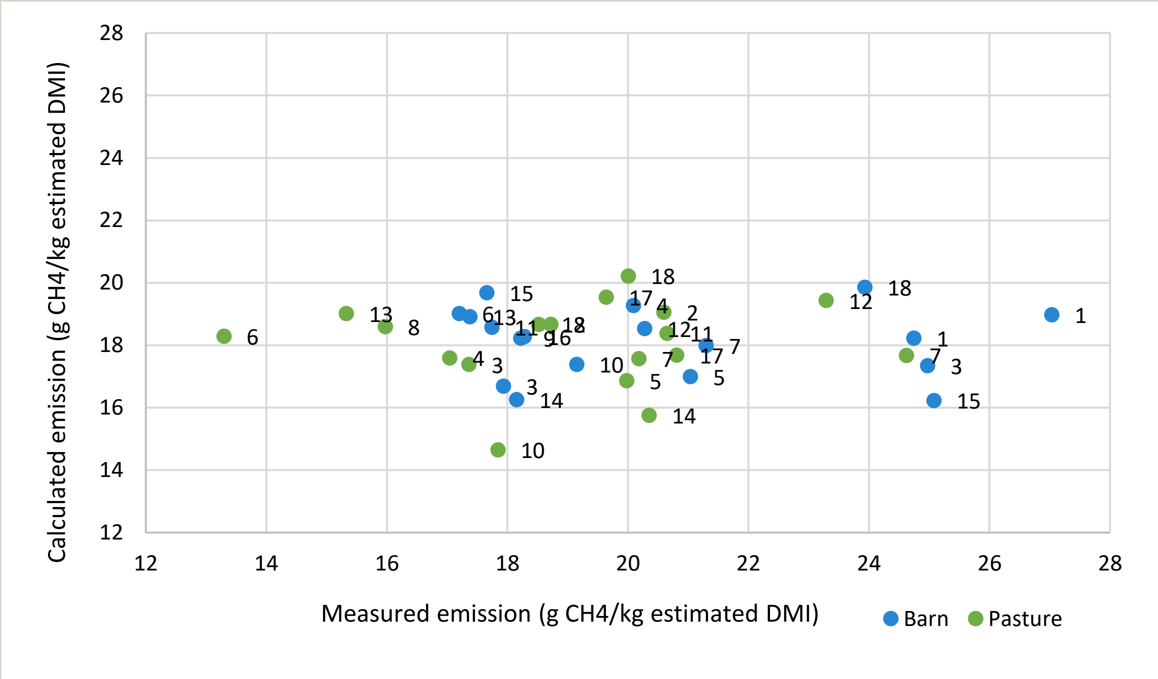


Appendix 6.2 *Barn and pasture records of all cows from farm 8 in one week of the two week measuring period in April 2019 during grazing.*

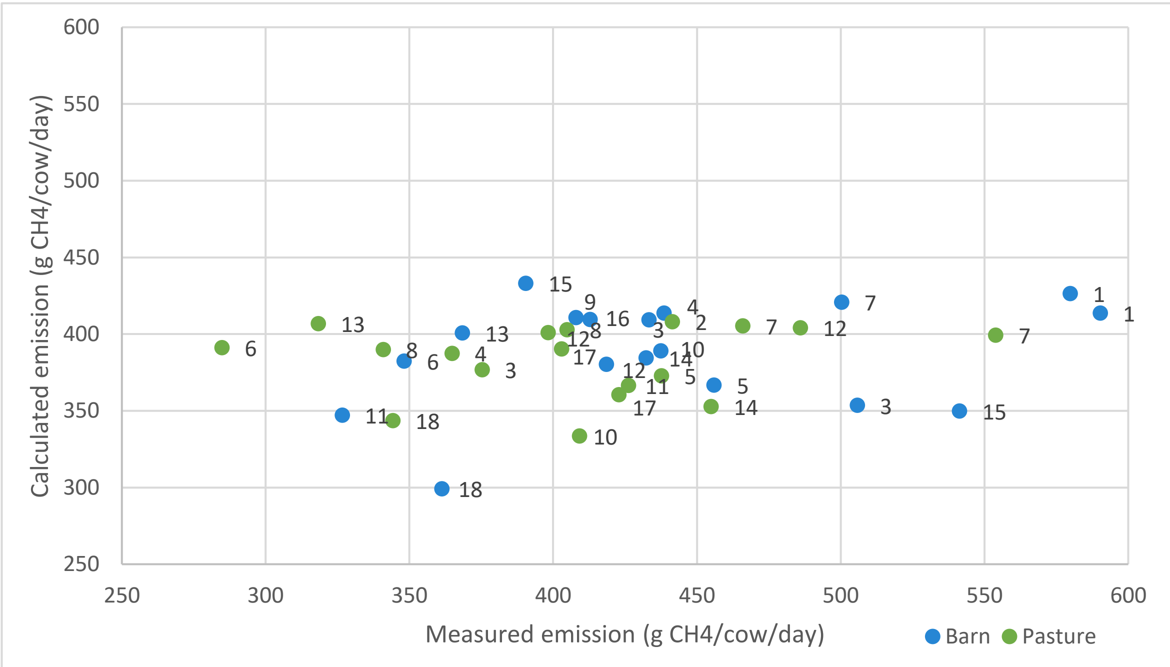


Appendix 6.3 *Barn and pasture records of all cows from farm 13 in one week of the two week measuring period in May 2019 during grazing.*

Appendix 7 Model calculations versus measured emissions



Appendix 7.1 *Calculated CH_4 yield (y axis) versus measured CH_4 yield (x axis) in g CH_4 per kg of estimated DMI. Each point is an average of the test group per farm per measurement (farm number is noted in the graph). In green the measurements during pasture season, in dark blue to measurements during barn season. The corresponding model is Predicted(Y)=18.72-0.033*X. The lack of fit is large (F=279.23, p<0.001).*



Appendix 7.2 *Calculated* CH_4 production *(y axis) versus measured* CH_4 production *(x axis). Each point is an average of the test group per farm (farm number is noted the graph) per measurement. In green the measurements during pasture season, in dark blue to measurements during barn season. The corresponding model is Predicted(Y)=346.4+0.0923*X. The lack of fit is large (F=178.02, p<0.001).*

To explore the potential of nature to improve the quality of life

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