Cécile Levrault

Practical monitoring of enteric methane production from individual ruminant

Propositions

1. Imperfect monitoring of the methane production from individual ruminants is better than no monitoring at all.
   (this thesis)

2. Striving for a “one size fits all” solution for monitoring enteric methane production of individual dairy cows in practice is an utopic enterprise.
   (this thesis)

3. Communicating about set-backs and failures in the scientific community is more important than publishing about successes.

4. Travelling across the world to attend conferences on greenhouse gas reduction is hypocritical.

5. Positive discrimination towards women in academia is anything but empowering.

6. The level of conviviality of a society is positively correlated with the daily fraction of time it devotes to food.

Propositions belonging to the thesis, entitled
Practical monitoring of enteric methane production from individual ruminants

Cécile M. Levrault
Wageningen, 16 January 2024

Practical monitoring of enteric methane production from individual ruminants

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Practical monitoring of enteric methane production from individual ruminants

Cécile M. Levrault

Thesis

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Dedicated to my parents,
for making all of this possible.

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Summary

Global anthropogenic activities contribute to increasing atmospheric concentration levels of greenhouse gases (**GHG**), mostly in the form of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and fluorinated gases (EPA, 2012). At the current GHG emission levels, this enhanced greenhouse effect has already and unequivocable started causing climate change, with a global surface temperature rise of 1.1°C from 1850-1900 to 2011-2020 (IPCC, 2023) and negative effects on the environment. It is therefore urgent and essential that the GHG emissions from anthropogenic activities drastically reduce.

Amongst these human-sourced activities, the ones related to agriculture (within the farm gate) themselves appear to be responsible for 9 to 14% of the total share (Mbow et al., 2019). The non-CO_2 emissions from livestock were estimated by Herrero et al. (2016) to range between 2.0 and 3.6 Gt CO_2-equivalents per year, with cattle being the main source of emissions from the sector with 65-77% of the share (FAO, 2021a; Mbow et al., 2019). Considering that livestock's emissions are for 40% due to methane resulting from enteric fermentation (Vonk et al., 2018), reducing its production (essentially by cattle) is one of the priorities. However, evaluating the performance of mitigation strategies requires techniques to monitor the enteric methane production of individual animals. Therefore, this thesis explores possibilities to monitor enteric methane production, at individual level and under practical conditions.

Chapter 1 provides an overview of the current need of reducing anthropogenic GHG emissions. Attention is given to the role of agriculture and livestock, with a focus on the emission of methane by ruminants. The processes leading to its production, as well as diverse reduction strategies are presented. Subsequentially, the available methods for monitoring enteric methane production at individual level under both controlled and practical conditions are described. Challenges and limitations emerging from practical measurements are highlighted. The aims of the thesis are delimited and defined as:

- Develop an improved version of the cubicle hood sampler and test the performance of its embedded sensors.
- Investigate the ability of two practical devices (portable accumulation chamber and cubicle hood sampler) to assess methane production rates in sheep and cows respectively.
- Develop a model allowing to assess postprandial methane production at population and individual levels, in order to convert discrete measurements into daily production rates.

- Report and discuss the challenges and limitations encountered when monitoring enteric methane in ruminants under practical conditions, and provide perspectives.

To address these aims, a first practical monitoring device is introduced in **Chapter 2**. This so-called portable accumulation chamber (**PAC**) consists in an airtight compartment in which small ruminants (*e.g.* sheep, goats) are held for about an hour. The methane and carbon dioxide produced by the animal accumulates in the chamber (no ventilation) before being sampled and the methane production rate (**MPR**) determined from the measured increase in concentration and air volume. Literature research showed that this device is already used around the world, but its performance in terms of absolute measurement accuracy has not been tested. Using a mass flow controller (**MFC**) releasing known masses of methane, we investigated the ability of a set of ten PAC to (repeatedly) measure absolute methane production levels, and to rank animals according to them. After excluding two severely leaking chambers, this study showed that the measurements made by the remaining eight PAC were highly repeatable across chambers and replicates. However, methane recovery appeared to be under-performing by roughly 30%, and to be significantly decreasing with increasing injected mass. We suspect both the under-recovery and the drift to be due to measurement errors made by the gas analyser as it was not calibrated. The results of this study emphasize the necessity of whole-system calibrations when monitoring gaseous exchanges. We could conclude that the tested set of PAC can be used to rank animals based on their MPR but should not yet be used for studies requiring high measurement accuracy.

Measuring enteric methane production in practice is (currently) only possible through spot sampling. Knowing that methane production is generally non-linear and dependent of feeding regime and time of the day, we formulated a model allowing to convert non-continuous measurements into postprandial production curves. This hierarchical methane rate (**HMR**) model consists in a Bayesian hierarchical stochastic kinetic equation, and is presented in **Chapter 3**. In this chapter, the model was used to fit a non-linear curve on the climate respiration chamber (**CRC**) dataset of twenty-eight lactating cows before computing an area under the curve, thereby providing an estimate of MPR from individual cows. The shape parameters of the model were pooled across cows (population-level), while the scale parameter varied between individuals. This allowed for the characterization of variation in MPR within as well as between cows. In this chapter, model fit was thoroughly investigated. We concluded that the MPR predictions made by HMR for these cows appeared to reflect individual MPR

levels and variation between cows as well as the standard approach taken by scientists with CRC data.

In **Chapter 4**, the design of the latest cubicle hood sampler (**CHS**) prototype is presented. This system aims at monitoring the individual MPR of dairy cows on-barn while animals lie down in cubicles with CHS. The air exhaled by the animal is collected by the device so that its methane content can be evaluated. After detailing the specificities of this prototype, embedded sensors intended to identify animals and monitor their head position are presented. The data collected by the ultrasonic sensors did not allow to monitor head positions as the sensors kept failing. Identifying animals using radio frequency identification (**RFID**) first yielded satisfactory results, but resulted in a failure of the system. Subsequently tested in a new setting, sufficient levels of correct identification could no longer be reached. In addition, recovery tests were conducted using an artificial reference cow (**ARC**) to assess the ability of the CHS to monitor two levels of MPR. The recovery rates obtained indicated the ability of the system to estimate these two production rates.

Chapter 5 investigates the ability of the CHS to monitor the MPR of twenty-eight lactating dairy cows. For this, the individual MPR of the animals were consequently assessed by the gold standard CRC and the CHS. Due to the system failures detailed in **Chapter 4**, CHS data was only obtained for twenty-one cows. The daily MPR estimated by the two devices for these animals were compared, which showed their poor correlation. Ranks were computed according to the production levels, which resulted in an acceptable level of agreement. After that, the eleven cows with the most CHS observations (>30) were selected. An independent CRC dataset (seventeen cows) was fitted with HMR, thus providing informative prior distributions for the hyperparameters used in the eleven cows CHS fit. New linear regression of the MPR levels estimated by the CRC and the CHS for these eleven cows showed that using HMR did not improve correlation levels nor ranking. However, the results obtained in this chapter enabled the detection of a persistent bias in the estimations made by the CHS. We suspect it to be due to a poor recovery of the breath sample by the device, and to errors made in the monitoring of background methane concentrations. The model could not compensate for this bias, whose cause(s) must be identified and resolved. In its current state, the CHS should not be used to measure absolute methane production levels, but it can be used (with care) to rank animals.

Lastly, **Chapter 6** concludes and discusses the practical implications of the research conducted in this thesis. The challenges encountered when trying to monitor enteric methane with two spot sampling devices are summarized. The key factors that appear to play an important role in

measurement accuracy are identified. They consist in the recovery of the breath sample and its representativeness, the representativeness of the background measurements, the performance and accuracy of the whole-system being used to monitor MPR, and finally the overall sampling scheme and data analysis. Possible solutions to these challenges and suggestions for further outlook are proposed. Overall, this work has brought to light the complexity of monitoring enteric MPR in practice, and the interconnectedness of the impacting factors. It is strongly recommended to address the key elements in a systemic manner with respect to their interconnectedness as demonstrated in this thesis.

Nomenclature

Abbreviations

ARC	Artificial reference cow
AUC	Area under the curve
CCC	(Lin's) Concordance correlation coefficient
CHS	Cubicle Hood Sampler
CH_4	Methane
CRC	Climate respiration chamber
CO_2	Carbon dioxide
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ELPD	Expected log pointwise predictive density
FTIR	Fourier-transform infrared spectroscopy
GEM	GreenFeed emission monitoring
GHG	Greenhouse gas
GLM	Generalized linear model
GWP	Global warming potential
HMR	Hierarchical methane rate
HTS	Cubicle hood sampler training set
ICC	Intraclass correlation coefficient
IR	Infrared
LMD	Laser methane detector
LOO-CV	Leave-one-out cross validation
MC	Measurement cycle

MFC	Mass flow controller
MPR	Methane production rate
OHS	Original cubicle hood sampler (data)set
ORS	Original climate respiration chamber (data)set
PAC	Portable accumulation chamber
PPC	Posterior predictive check
PPD	Posterior predictive distribution
RFID	Radiofrequency identification
RMSE	Root mean square error
RT	Recovery test
RTS	Climate respiration chamber training (data)set
TMR	Total mixed ration
ULS	Ultrasonic sensors

Symbols

α	Scale parameter of the HMR equation
β, γ and δ	Shape parameters of the HMR equation
σ	Variance
θ	Cow head-body angle (degrees)
CH_4	Methane
$[CH4]_B$	Background methane concentration (ppm)
$[CH4]_H$	Hood methane concentration (ppm)
CO_2	Carbon dioxide
CV	Coefficient of variation (%)
H_2	Hydrogen
m	Methane concentration (mg/m^3)
M	Molar mass of methane ($kg.mol^{-1}$)
N_2O	Nitrous oxide
O_2	Oxygen
p	P-value
P	Ambient pressure (mbar)
Q	Airflow (m^3/h)
r	Pearson's correlation coefficient
R	Universal gas constant ($J.mol^{-1}.K^{-1}$)
R^2	Coefficient of determination
SF_6	Sulphur hexafluoride
T	Ambient temperature (K)
V_M	Molar volume of methane ($m^3.mol^{-1}$)

Chapter 1.

General introduction

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1.1. Man-induced climate change and the role of agriculture

Global anthropogenic activities contribute to increasing atmospheric concentration levels of greenhouse gases (**GHG**), mostly in the form of carbon dioxide (**CO_2**), methane (**CH_4**), nitrous oxide (**N_2O**), and fluorinated gases (EPA, 2012). By absorbing and reflecting infrared light, these gases affect the solar energy outflow of the earth and create a greenhouse effect. Energy that is emitted by the sun essentially enters our atmosphere as short wave radiation (visible light). When leaving it, due to reflection from the earth surface, it does so as infrared (**IR**) radiations, which correspond to wavelengths that are longer than the visible section of the red spectrum, and shorter than microwaves. These IR radiations are perceived as heat. When present in the atmosphere, GHG reflect back to Earth part of the IR radiations that should have left it, thus trapping heat into the atmosphere (UNFCCC, 2007). At the current GHG emission levels, this enhanced greenhouse effect has already and unequivocable started causing climate change, with a global surface temperature rise of 1.1°C from 1850-1900 to 2011-2020 (IPCC, 2023) and negative effects on the environment. It is therefore urgent and essential that the GHG emissions from anthropogenic activities drastically reduce.

However, the reported levels of average annual GHG emissions by the Intergovernmental Panel on Climate Change (IPCC) between 2010 and 2019 were found to be higher than in any previous decade. All together, they have been estimated to represent a total net emission of 59 ± 6 Gt CO_2-equivalents in 2019, which is 12% higher than the level of 2010 (IPCC, 2023).

Amongst these human-sourced activities, the ones related to agriculture (within the farm gate) themselves appear to be responsible for 9 to 14% of the total share (Mbow et al., 2019). Using the global warming potential (**GWP**) values to represent the energy absorbed by each gas over a 100 years and in comparison with CO_2 (Myhre et al., 2013), this corresponds to a total mass of 6.2 ± 1.4 Gt CO_2-equivalents that is emitted by agricultural activities (IPCC, 2013). During the period going from 2007 to 2016, crop and livestock productions generated 142 ± 42 Tg CH_4 and 8.0 ± 2.5 Tg N_2O per year (Mbow et al., 2019). Their respective GWP of 28 and 265 at a 100 year horizon (Myhre et al., 2013) are not neglectable.

With numbers varying slightly between references, the non-CO_2 emissions from livestock (within farm gate) were estimated by Herrero et al. (2016) to range between 2.0 and 3.6 Gt CO_2-equivalents per year, and by the FAOSTAT (2018) to have been around 4.1 ± 1.2 Gt CO_2-equivalents yearly between 2010 and 2016. Independently of the inclusion or exclusion of outside-the-farm-gate parameters (*e.g.* energy and land use, transportation), all references agree

that cattle is the main source of global emissions from the livestock sector, with 65-77% of the share (FAO, 2021a; Mbow et al., 2019). Small ruminants such as sheep and goat are for their part responsible for about 6% of the share, with an estimated yearly emission of 0.47 Gt CO_2-equivalent (FAO, 2013). Even though the GHG emissions per unit of animal product produced (GHG emissions intensity) have globally largely declined (approximately 60% lower in the 2010s than in the 1960s) thanks to improved milk and meat productivity of cattle (Davis et al., 2015; FAOSTAT, 2018), the general GHG emissions trend from livestock continues to be upwards due to the increasing demand for livestock products (Mbow et al., 2019). Considering that livestock's emissions are for 40% due to methane resulting from enteric fermentation (Vonk et al., 2018), and for 9% (5% N_2O, 4% CH_4) due to manure and its management (FAO, 2014), reducing the methane emissions (especially the enteric methane produced by cattle) is one of the priority reduction pathways to be investigated.

1.2. Methane emissions from cattle: origins and reduction strategies

Methane emitted by cattle originates from two sources: the rumen (enteric fermentation), and the manure (biological and chemical processes occurring after excretion). These are described below.

1.2.1. Enteric methane

Methanogenesis

The first process occurs once a ruminant has ingested feed (postprandial) that is taken to the anaerobic, methanogenic environment of the digestive tract. There, and more particularly in the rumen, the organic matter contained in the alimentary bolus is degraded by a diverse community of anaerobes during what is called the anaerobic fermentation process. First, the primary anaerobic fermenters convert the structural carbohydrates, proteins and other organic polymers of the plants that are contained in the feed into their monomer components (Figure 1.1). These monomers are then converted by the primary fermenters and other microbes into volatile fatty acids, H_2 (hydrogen) and CO_2. These latter products of fermentation (H_2 and CO_2) are then used as main substrate by the methanogens to produce CH_4 (Morgavi et al., 2010). Ruminal fermentation rate is time dependent, with postprandial durations affecting methane production rate (**MPR**) in a non-linear manner. A rapid rise towards a methane production peak (reached 30 to 140 min after feeding) and a slow decrease back to the basal production level (Crompton

et al., 2011; van Lingen et al., 2017) can be observed in cattle, the amplitude and period depending mainly on the feed intake pattern.

Generally, methane is perceived as not only a potent GHG, but also as an energy loss for the animal (reduction of the feed conversion efficiency) and an economic waste for the farmer (González-Recio et al., 2020). Nevertheless, it is important to note that it plays a crucial role in maintaining the H_2 levels to quantities that do not inhibit the normal functioning of the microbial enzymes involved in the digestion process of fibrous feed (Morgavi et al., 2010).

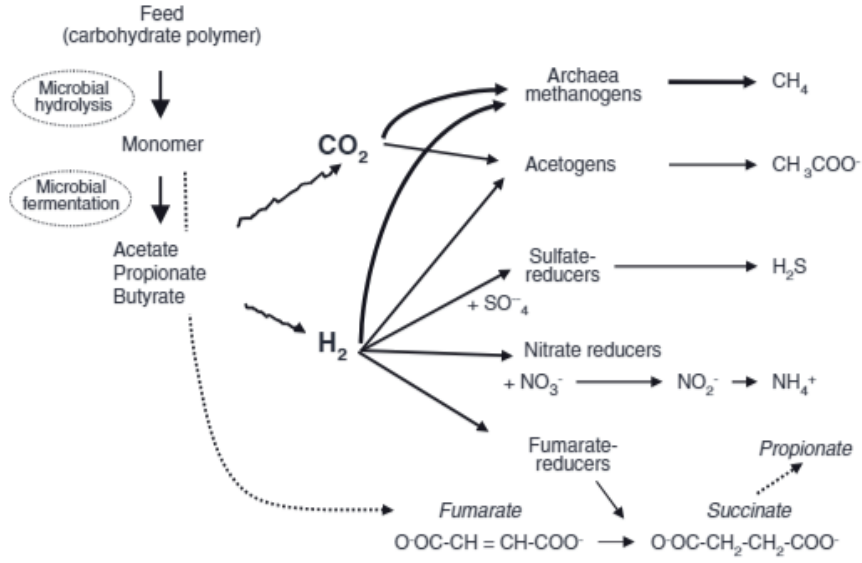

Figure 1.1. Schematic microbial fermentation of feed polysaccharides and H_2 reduction pathways in the rumen (Morgavi et al., 2010).

Mitigating enteric methane production

Diet

The main driver for enteric methane production has long been known to be the diet. Therefore, changing the ration's content is the most effective straightforward approach to reduce enteric methane production (Haque, 2018; Kebreab et al., 2010). Forage and concentrate qualities, contents and ratios can be altered. For example, ensuring a lower content of non-digestible fibre increases both digestibility and passage rate, therefore redirecting rumen fermentation towards propionate (Hills et al., 2015). As shown by Beauchemin et al. (2009), propionate serves a role

as an H_2 sink, meaning that an increased propionate content in the rumen leads to lower amounts of H_2 available for methanogenesis. Starch content in the diet can also be increased, for example through the implementation of maize in the ration (Hart et al., 2015). At it is low in protein, and its starch content partially bypasses the rumen, the production of methane by the methanogens is reduced. Still, its digestion in the small intestine provides the animal with the necessary energy, notably in the form of glucose. Feeding maize has the simultaneous advantage of improving nitrogen efficiency, leading to lower presence of unutilized nitrogen in the urine (Dijkstra et al., 2013; Hristov et al., 2013a). Another possibility is to increase the share of concentrate in the provided ration. While the increase in dry matter intake (DMI) does lead to an increase in absolute methane production (g/d) (Boadi and Wittenberg, 2002; Lovett et al., 2005; van Wyngaard et al., 2018), it still decreases both CH_4 intensity (g CH_4 per kg of output product) (Aguerre et al., 2011; Yan et al., 2010) and CH_4 yield (g CH_4 per kg of DMI) (Jiao et al., 2014; Tyrrell and Moe, 1972).

Another approach to be taken with feeding is the inclusion of additives in the ration. They usually are based on organic acids, chemical inhibitors, secondary plant compounds (Knapp et al., 2014), or probiotics (Haque, 2018), that either directly inhibit methanogens (or methanogenesis), favour H_2 disposal, or supress ciliate protozoa (Knapp et al., 2014). In any case they must be used with care as too extensive defaunation is correlated with an increase in ruminal methanogen density (Mosoni et al., 2011), and too high concentrations of H_2 can lead to the inhibition of the fermentation process (Weimer, 1998).

Breeding

Reducing enteric methane production can also be achieved by breeding lines of animals that naturally and permanently produce less methane (Boadi et al., 2004). Indeed, several studies demonstrated the presence of significant variation in methane production between animals, in both sheep and cows, in relation with phenotypic traits and heritability (Breider et al., 2019; Lassen and Løvendahl, 2016; Pinares-Patiño et al., 2003). This approach has the key advantage of permanently reducing the methane production of the animals. However, breeding individuals with lower methane production levels may be incompatible with other breeding objectives (Eckard et al., 2010). Genetic selection that improves resistance, health, and fertility of the descendance will lead to increased lifetime and productivity, therefore indirectly reducing CH_4 intensity (Knapp et al., 2014).

Lastly, herds can be managed differently so that overall (lifetime) productivity is increased (Boadi et al., 2004; Johnson et al., 1996; Moss et al., 2000). One approach is to replace non-productive and low-producing animals with high-producing ones. Maintenance of the latter will increase the total net methane production of each animal, but their CH_4 intensity will decrease (Gebbels et al., 2022; Patra, 2012). This approach must be supported by adequate feeding management to improve productivity and reduce CH_4 intensity (Haque, 2018).

Other farm management strategies

Additionally, the number of dry cows and replacement heifers should be kept to a minimum, which stems from the need for an increase of the animals' life expectancy, and the reduction of the culling rate (Knapp et al., 2014). Nevertheless, replacement levels that are too low can compromise genetic progress and should be avoided (Gill and Allaire, 1976).

Strategies to increase fertility rate may also be implemented (Smith et al., 2014). Indeed, lower fertility rates and increases of days open (number of days a cow is not pregnant) lead to lower reproductive efficiency, lower productive lifetimes, and therefore to higher CH_4 intensity (Knapp et al., 2014). Higher fertility can be achieved by ensuring the good health and comfort (*e.g.* avoidance of heat stress) of the animals (Hansen, 2007; Kadzere et al., 2002), and implementing optimal reproduction programs (Knapp et al., 2014).

A recapitulative overview of the enteric methane mitigation strategies can be found in Figure 1.2 (Arndt et al., 2022).

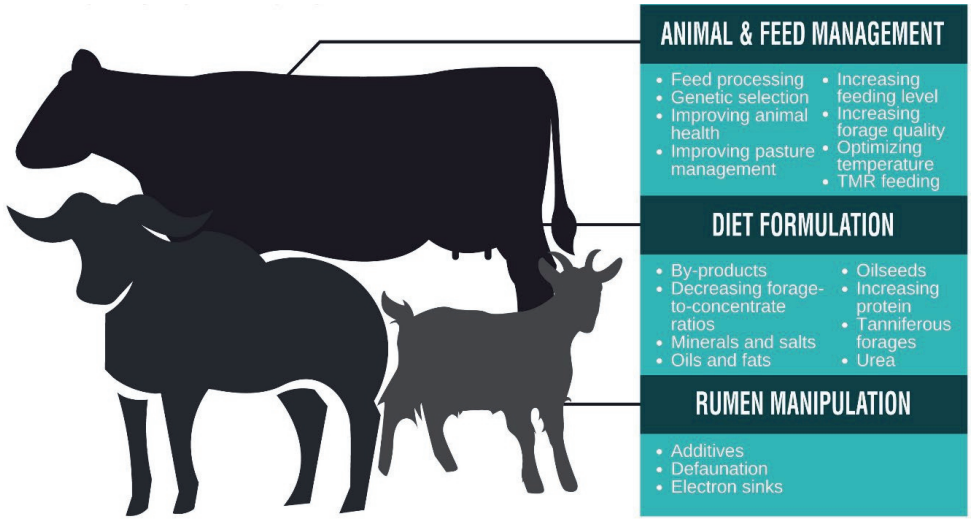

Figure 1.2. Enteric methane mitigation strategies in ruminants (Arndt et al., 2022).

1.2.2. Methane from manure

Manure composition and methane production

Faeces excreted by ruminants are essentially composed of nitrogen (mostly in its inorganic form), carbon, and water (Chadwick et al., 2011; Paul et al., 1993). These compounds are part of the essential factors leading to the production of CH_4 and N_2O in excreted manure. Methane, for its part, is generated during the anaerobic decomposition of the organic matter that is present in faecal matter, and in the bedding material (Batstone et al., 2002; Hellmann et al., 1997; Møller et al., 2004). Acid producing bacteria degrade these organic compounds into other compounds, such as volatile acids. Subsequentially and in the absence of oxygen, (methane producing) bacteria use these volatile acids to produce CH_4. Environmental factors, such as ambient temperature (Clemens et al., 2006; Sommer et al., 2007), manure composition, and manure management and storage (Hill et al., 2001; Ni et al., 2008) highly influence the extent to which methane is produced from the excreta.

Mitigating methane production from manure

A first lever for mitigation is the storage of manure in cooler environments (*e.g.* outside storage in cool climates), as lower temperatures slow down the methanogenesis process (Sommer et al., 2004; Umetsu et al., 2005). When ambient conditions are not cool enough, slurry channels can actively be cooled. If the exchange energy is functionally used, this can be a cost effective approach (Sommer et al., 2004). In addition, solid manure can be composted so that its degradable organic matter is converted by microorganisms into CO_2 and water. However, composting results in losses of nitrogen in various forms (Chavez-Rico et al., 2022). Occurring in (primarily) aerobic environments, this process decreases methanogenesis (Amon et al., 2001). Manure (in all forms) can also be covered during storage, with straw or plastic sheets (Chadwick, 2005; VanderZaag et al., 2009). In the case of slurry, the solid and liquid fractions can be separated using mechanical separation processes (Burton, 2007), or innovative barn floors (Galama et al., 2020). Separate storage of the two fractions results, in most cases but not all, in lower methane emissions (Dinuccio et al., 2008; Fangueiro et al., 2008). Chemical additives may also be added into the slurry storage to prevent the formation of CH_4 (Petersen et al., 2013).

1.3. Individual monitoring of enteric methane production

To evaluate the performance of the aforementioned reduction strategies, techniques are needed to monitor their effect on the methane production of the animals. In this thesis, focus is given to the reduction of the enteric emissions as it represents the main part of methane emissions (ca. 40%) in the agricultural sector (see section 1.1). While the effect of nutrition on enteric methane production can be studied at both individual and herd level, mitigation strategies such as genetic selection require individual monitoring (González-Recio et al., 2020). Additionally, they require repeated recordings of a large number of animals (Manzanilla-Pech et al., 2021), hence the need for monitoring under practical conditions (on farms). Consequently, only the available devices that measure the enteric methane production of individual ruminants will be presented.

1.3.1. Flux measurement methods

The first approach to measuring enteric methane production is with flux methods. The flux of a quantity can be defined as being the flow rate of that quantity, across a specific area and period of time (Stauffer, 2006). It therefore yields a trend that represents the dynamics of the production process.

Closed monitoring devices

Climate respiration chamber

The climate respiration chamber (**CRC**) has long been recognized as being the gold standard for measuring respiratory exchanges and gas production from animals, and is used to benchmark other methods. They consist of airtight compartments in which individual animals are kept for a period of time (Figure 1.3), usually ranging between 1 and 7-d (Hammond et al., 2016a). They vary in design and dimension between institutes and the species they are made for (GlobalResearchAlliance, 2018).

In the open-circuit CRC, inflowing air is circulated through the chamber to mix inlet air with the gases produced by the animal. The concentration of all gases of interest (*i.e.* CH_4) are measured in the inlet and the outlet air by a series of gas analysers, and the difference attributed to the animal. Methane emission is then determined by multiplying the concentration difference with the airflow rate (Kuhla et al., 2015). Measurements made in CRC are systematically

corrected for humidity, temperature, and pressure (standard temperature, pressure, dry, **STPD**) as they affect gas volume (Hammond et al., 2016a). In the facility of Wageningen University & Research, they allow for one outcome value every 10 min, being the average of samplings of 120 sec each (Alferink et al., 2015).

The CRC is the device with the most controllable environment, in terms of ambient conditions and air fluxes, therefore resulting in the most accurate MPR estimates. However, critical sources of variation remain, which are the air mixing, the airflow (in the chamber and the ducting system), and the gas analyser errors (Gardiner et al., 2015). In their study, Gardiner et al. (2015) showed that there were significant differences in recovery rate between twenty-two chambers across six facilities in the UK. Variation in recovery appeared to be due to uncertainties in the sample ducting and flow measurements (15.3%), the chamber mixing (3.4%), and in the methane analyser records (1.3%). This work brought to light the need for calibration and recovery testing, and the reporting of these results, which was emphasized by Gerrits et al. (2017).

By being highly precise, CRC are ideal for small scale experiments, in which the required accuracy and precision levels are high (Lassen and Difford, 2020). However, this methodology is expensive, labour intensive and has low throughput, which is prohibitive to measure large numbers of animals under experimental settings (Garnsworthy et al., 2019). Furthermore, the prolonged confinement within the chamber restricts natural behaviours and induces social isolation which could result in behavioural changes, reduced feed intake and performance (Gastelen et al., 2015). Reduction of the ingested quantities could result in an underestimation of the animal's methane production rate as feed intake is the primary driver of CH_4 production by ruminants (Hristov et al., 2013a; Kristen A Johnson and Johnson, 1995). These are limiting points for CRC to be used at large scale and in practical conditions.

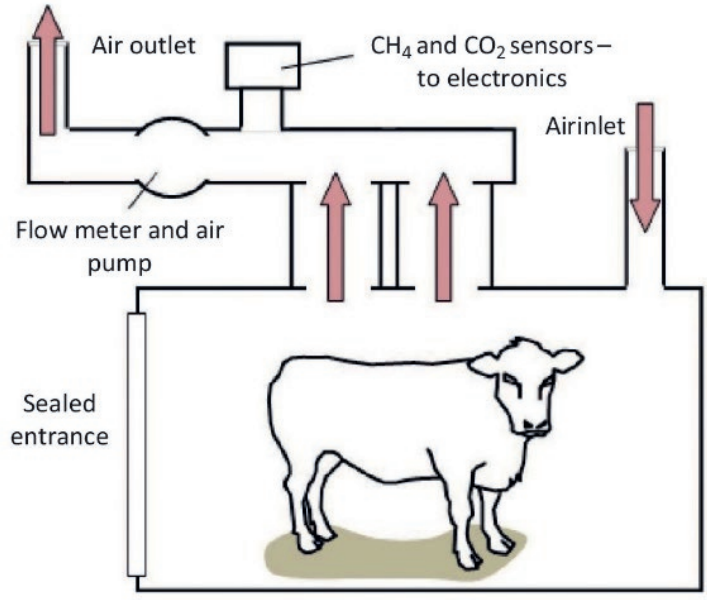

Figure 1.3. Schematic of a climate respiration chamber (Hill et al., 2016).

Portable accumulation chambers

Portable accumulation chambers (**PAC**) are an alternative to respiration chambers. Significantly less complex in their functioning, they are simple airtight boxes in which an animal is held for a period of 40 to 120 min (usually 50) (Jonker et al., 2020b). During this time, gases emitted by the animal accumulate in the chamber, while oxygen level depletes (Goopy et al., 2011; Hegarty, 2013). Changes in gaseous concentrations are recorded, usually in the beginning (to account for background concentrations), and the end of the experiment. The volume of the chamber, corrected for the estimated volume of the animal, is then used to estimate the mass flux of (methane) gas produced by the animal (Jonker et al., 2020b). They offer a low cost and higher throughput alternative to the CRC (Goopy et al., 2011).

The portable nature of the PAC has enabled researchers across the world to record CH_4 production from thousands of small ruminants, primarily for ranking of animals and selection experiments (Jonker et al., 2020b). However, there is a large variation in design of PAC across research groups and limited consensus on best practices (Jonker et al., 2020b) which may lead to unequal performances. The majority of the PAC validation studies involved recording sheep in respiration chambers and then recording with PAC to determine the correlation between both devices' estimates. For example, Goopy et al. (2011) found the methane estimates of PAC and

CRC to have a correlation of 0.71, while Robinson et al (2015) reported correlations between 0.0 and 0.19 when adjusted for liveweight and feed intake. In any case, sheep cannot be recorded by both methods simultaneously for logistical reasons, which poses a challenge to researchers to determine if differences between measurements with both methods are due to true differences in the methodologies or as a result of extraneous biological factors, such as intra or inter-day(s) variations, feed intake or dietary changes between measurements (Jonker et al., 2020b; O'Connor et al., 2021b, 2021a).

Additionally, PAC cannot be used over extensive periods of time to avoid negative effects which might result from the increasing concentrations of CO_2 in the chamber. They also only allow for a single spot sample per animal and per day of testing (Hammond et al., 2016a). Considering that MPR varies through time and in response to feeding, we can only assume that single spot samplings do not accurately reflect the daily MPR level of an animal, as the moment of sampling will determine the production rate and impact the extrapolated daily estimate. Lastly, animals that are monitored in sequence will not all be monitored at the same phase of the postprandial production curve, which adds to the variability between estimated levels. Only repeated measurements, randomly spread over time, could compensate for this type of error. The usage of PAC should therefore still be homogenized and optimized.

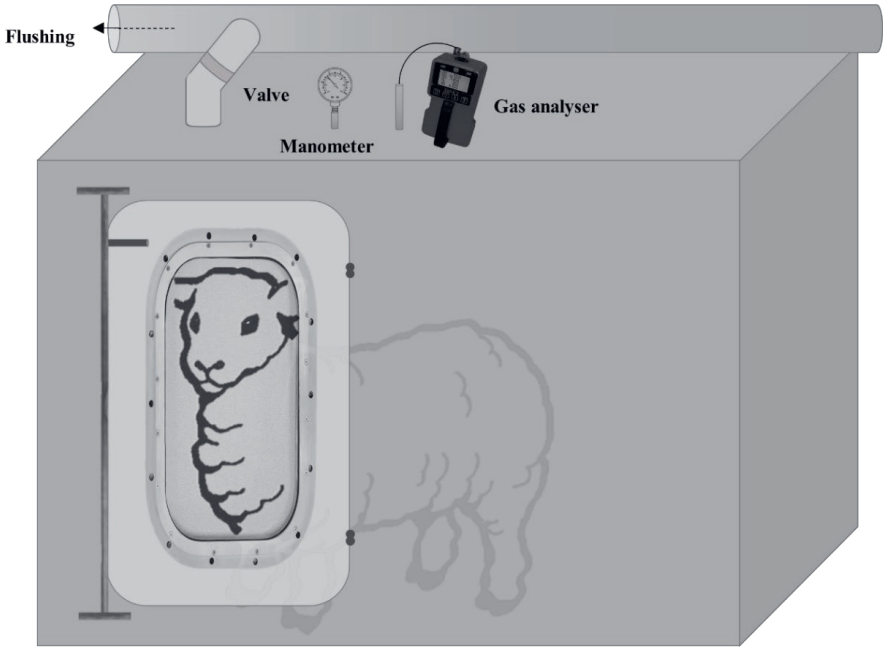

Figure 1.4. Schematic of a portable accumulation chamber.

Semi-closed monitoring devices

GreenFeed

The so-called GreenFeed Emission Monitoring (**GEM**) system (C-Lock Inc., South Dakota) is a stand-alone head chamber that can be placed anywhere in the pastures or the barns (Figure 1.5). It is programmed to distribute small amounts of concentrate at specific time intervals, with the aim of attracting animals into to the system. The system, equipped with radio frequency identification (**RFID**), identifies each animal upon arrival and times the duration of its presence. If an animal remains present with its head acceptably placed within the system for a minimum of 3 min, the concentration of CH_4 and CO_2 in its breath will be monitored by the nondispersive infrared sensor (Tedeschi et al., 2022) for about 3 to 7 min (Hammond et al., 2015). The concentration levels monitored are differentiated from the background concentrations to distinguish the production of the animal from the ones of other sources. This information is then connected to the airflow going through the system (which is induced by a fan and monitored by an anemometer) so that a flux can be calculated for each visit. The fluxes are then averaged over the measurement period to obtain a daily mean MPR (Della-Rosa et al., 2021; Tedeschi et al., 2022).

However, it is known that the confidence level of a MPR estimate is dependent on the number of samples taken for each animal (Cottle et al., 2015), their duration (Arthur et al., 2017), and their distribution within a 24-h period (Della-Rosa et al., 2021). There seems to be no consensus on the recommendations made in the literature regarding the number of visits required and their distribution over time (Gunter and Bradford, 2017; Hristov et al., 2015; Manafiazar et al., 2016), nor on how to analyse and average the collected data (Hegarty, 2013; Manafiazar et al., 2016). Repeatability and accuracy of the MPR estimated by the GEM seem to differ as well, as a result of the experimental design, the sample size, and the (reference) monitoring device their output is compared to. For example, Huhtanen et al. (2019) found GEM measurements to be highly correlated to CRC ones ($r > 0.90$), while Hammond et al. (2015) found them to have poor concordance with the levels estimated by the gold standard. In the same study however, they found GEM estimates to have moderate concordance with the levels measured with the sulphur hexafluoride tracer gas method. For their part, Alemu et al. (2017) found CH_4 estimates to differ between GEM and CRC, but concluded that MPR can be accurately estimated by the GEM when time of feed intake is known. Optimally, full system calibration of the GEM system should be done to conclude if these diverging results are the consequence of varying experimental designs, frequency and number of samples taken, or are due to measurement

errors that lie within the system. A calibration study conducted by McGinn et al. (2021) with a mass flow controller (**MFC**) releasing known masses of CH_4 into a GEM placed in a CRC allowed the authors to conclude that the GEM can accurately estimate CH_4 emission rate over a short measurement period. However, the authors stipulated that their study neglected the additional errors that are known to occur when determining the 24-h MPR of an animal (Velazco et al., 2016), which may lead to different results in practice.

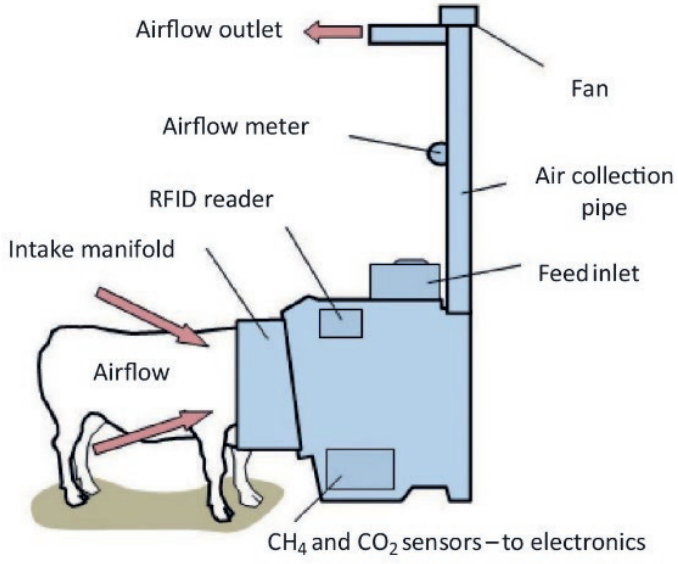

Figure 1.5. Schematic of a GreenFeed emission monitoring system (Hill et al., 2016).

Cubicle hood sampler

The cubicle hood sampler (**CHS**) was first developed by Wu et al (2016). Placed in the cubicles of a dairy barn, it relies on the fact that cows lie down up to 12-h per day in a relatively stable position, therefore enabling extended monitoring durations. This makes the CHS an appealing method, as it could allow to overcome the effect short sampling times have on the MPR estimations of other alternative methods (Kuhla et al., 2015).

Two prototypes were designed and developed by Wu et al (2016). They both consist of three vertical panels isolating the space around the head of the cow, that are connected to an extraction hood. The hood is connected to a piping system at the end of which a fan extracts the air from the hood, with the aim of collecting all air exhaled by the cow present cow. The piping includes

a flow rectifier and a flow meter (enabling flux calculation). Its methane concentration (amongst others) is then monitored by a Fourier Transform Infrared (**FTIR**) spectrometer gas analyser. A fan, placed before the exhaust, induces a flow rate within the CHS (Figure 1.6).

In the first design, called "basic sample hood", the gas sampling of the hood consists in an open inlet (Figure 1.7). In the upgraded version (called "fume sample hood"), two additional panels (one vertical, one horizontal) are added inside the hood to reduce inlet size. Inlet area is thus limited to perforations points in the added panels, aiming to create a negative pressure under the hood, thus increasing the suction effect, while limiting dilution with background air. A complementary curtain is mounted at one end of the hood (behind the cow's shoulders) to decrease the hood openness (Figure 1.8).

The authors evaluated the ability of the fume sample hood to recover known fluxes of methane in a barn (basic design tested under laboratory conditions). These fluxes were induced by an artificial reference cow (**ARC**) that controls the methane release rate through its mass flow controller (**MFC**), and simulates the exhalation and eructation cycles of a cow. Regardless of the air velocity and ARC-to-inlet distance that were experimented with, the fume sample hood was able to recover 97.2 ± 8.1% of the methane released.

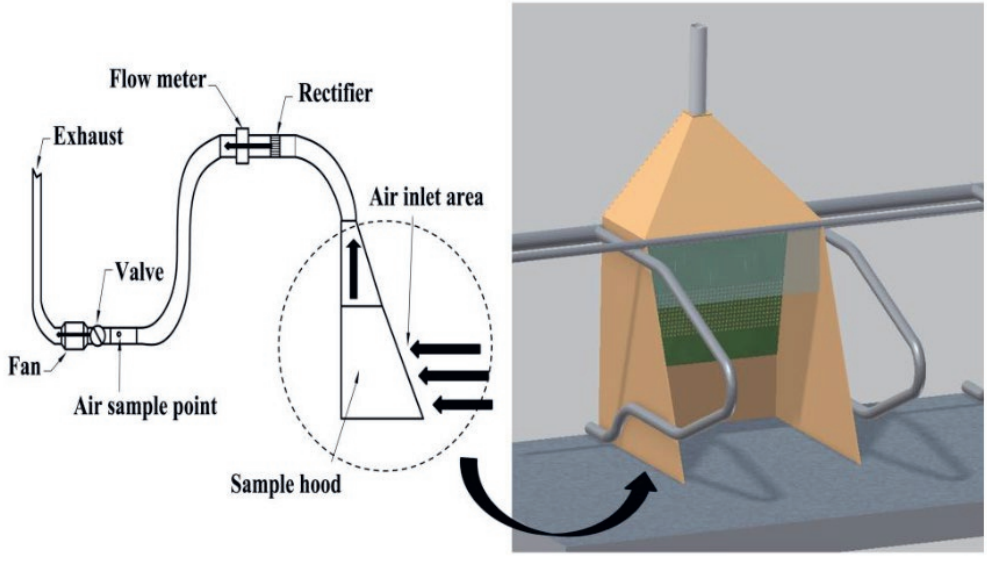

Figure 1.6. Schematic side view of the Cubicle Hood Sampler, with a fan creating a controllable airflow creating a negative pressure at the inlet, and a piping system equipped with a flow meter transporting the extracted breath air to the exhaust. Arrows indicate airflows. Positioning of the Cubicle Hood Sampler is shown on the right (Wu, 2016).

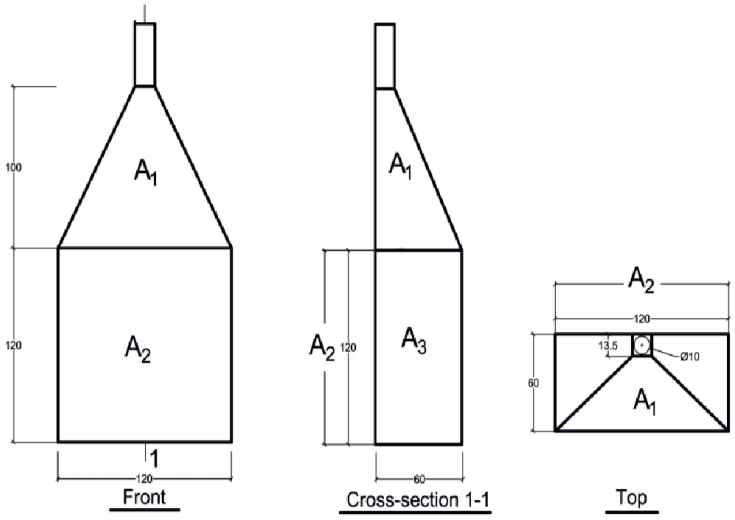

Figure 1.7. "Basic sample hood" prototype, showing the front, cross-section 1-1, and top view of the hood, composed of a top hood (A1), a front panel (A2), and two side panels (A3) (Wu, 2016).

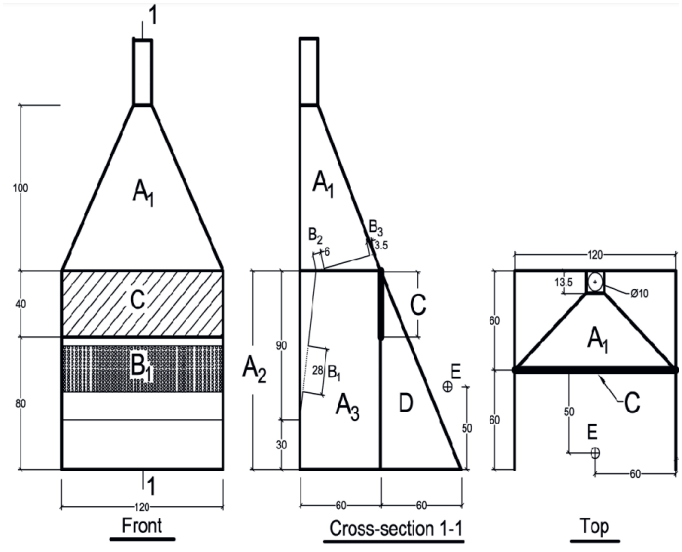

Figure 1.8. "Fume sample hood" prototype, showing the front, cross-section 1-1, and top view of the hood, composed of the same components as the basic sample hood with two additional panels, one placed vertically and one horizontally. The panels contained three gas inlets: at the front (B1), the middle top (B2), and the upper top (B3). Each gas inlet was made of a perforated panel with round holes. The fume sample hood was also extended with a top curtain (C) and two extended side panels (D).

Open-air monitoring device

Tracer gas measurements

Tracer gases are not flux methods per se, but consist in linking information on two fluxes. For this, the known release rate of a gas is linked to the unknown production rate of another gas, in this case methane. The most common case for measuring enteric methane is to use sulphur hexafluoride (**SF_6**) gas.

Its use was first reported by Johnson et al. (1994a), who assumed that the emission of SF_6 simulated the one of CH_4, with identical dilution rates. They thus stated that the MPR of a cow could be calculated by using the CH_4 and SF_6 concentrations measured in the breath air, together with the known release rate used for SF_6. They introduced a protocol in which a permeation tube (a threaded stainless steel tubing equipped with a cap) releasing a controlled rate of SF_6 (between 500-1000 ng of SF_6/min in their case) is placed in the rumen of a cow (Figure 1.9). The animal is simultaneously equipped with a collection vessel placed on its back or around its neck, and a capillarity tube going from the vessel to the head of the animal (close to the mouth and nostrils) where it is held in place by a halter. Samples of the exhaled air are continuously collected over a recommended period of minimum 5-d, covering full 24-h periods (Hammond et al., 2016a). Regular measurements of the background concentrations in SF_6 and CH_4 must be made so that they can be deduced from the concentrations found in the collection vessel (Williams et al., 2011).

A number of studies compared the MPR estimated for the same animals in CRC and with SF_6 as a tracer gas. In general, the SF_6 method leads to slight variations in the methane production that is estimated, which can oscillate between 5 and 10% lower or higher than the MPR estimated by the CRC for the same animals (Boadi et al., 2002; Grainger et al., 2007; Pinares-Patino et al., 2011). However, variation within and between animals was found to be considerably higher with the SF_6 approach than with the gold standard (Grainger et al., 2007; Pinares-Patino et al., 2011). Guidelines were published in 2020 to try to homogenize the use of this technique to measure enteric methane from ruminants (Berndt et al., 2020). By addressing issues such as the declining release rate of SF_6 through time (Deighton et al., 2013), or the necessary low background CH_4 and (residual) SF_6 concentrations (Berndt et al., 2020) could help reduce variability and increase repeatability and accuracy.

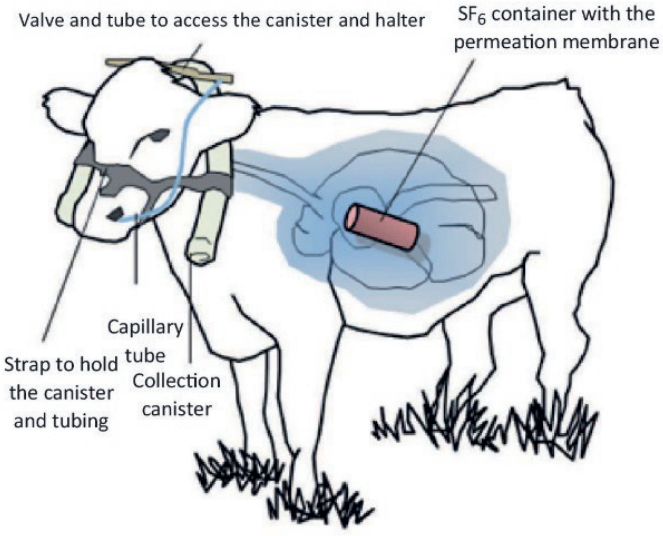

Figure 1.9. Schematics of the sulphur hexafluoride tracer gas technique (Hill et al., 2016).

1.3.1. Concentrations measurements methods

The second approach to monitoring enteric methane production is by evaluating the concentration of methane that is present in the breath sample. Devices that solely monitor concentration do not allow the calculation of methane production rates, but provide information on the quantity of gas that is present in a certain volume.

Semi-closed monitoring device

Sniffers

The use of sniffers was first presented by Garnsworthy et al. (2012) and corresponds to the eructation samples that are taking during feeding or milking. They can globally be characterized as sampling probes placed in automated milking systems (**AMS**) or in feeders that are directly connected to a gas analyser (Figure 1.10). Gas analysers used differ between experiments and facilities, which operate at varying sampling intervals and accuracy levels. An embedded software is used to identify and monitor eructation peaks (Hammond et al., 2016a). Usually, these peaks are monitored over visits of 3 to 10 min to be analysed as an overall mean, or as the mean of the detected eructation peaks (Garnsworthy et al., 2019). Eructation peaks can be converted into concentrations emitted by the animal by estimating the dilution rate of the

sampled eructed air. A value of the dilution rate can be obtained through regular calibrations (Bell et al., 2014).

Downsides associated with sniffers are linked to the fact that they solely monitor concentrations, and not fluxes, which do not allow to estimate production rates (Lassen and Difford, 2020). They also only detect and monitor eructation peaks, and do not take into account volume or rate of exhaled air nor flatulence (Garnsworthy et al., 2012) and are therefore not representative for all emissions. In addition, Hegarty (2013) and Huhtanen (2015a) have highlighted the influence of the distance between the animal's head and the inlet on the gas concentrations measured in exhaled air samples, with longer distances leading to lower concentrations measured and to biased estimates. Lastly, visit frequencies vary between animals, with respect to parity, feed intake, milk yield, and milking frequency. Dominances and competition for accessing the feeder or AMS are also likely to impact visit frequency and timing, and thus the resulting methane estimates (Hammond et al., 2016a; Lyons et al., 2014).

Presenting high levels of random errors and experimental variation (Huhtanen et al., 2015a; Huhtanen and Hristov, 2018; Wu et al., 2018), the use of sniffers has been excluded from national inventories and nutritional studies (Garnsworthy et al., 2019; Hristov et al., 2018), but they could maybe still be used in studies investigating genetic correlations, as sniffers measurements have been shown by some studies to be repeatable (Lassen et al., 2012) and heritable (Lassen and Løvendahl, 2016; Pszczola et al., 2019).

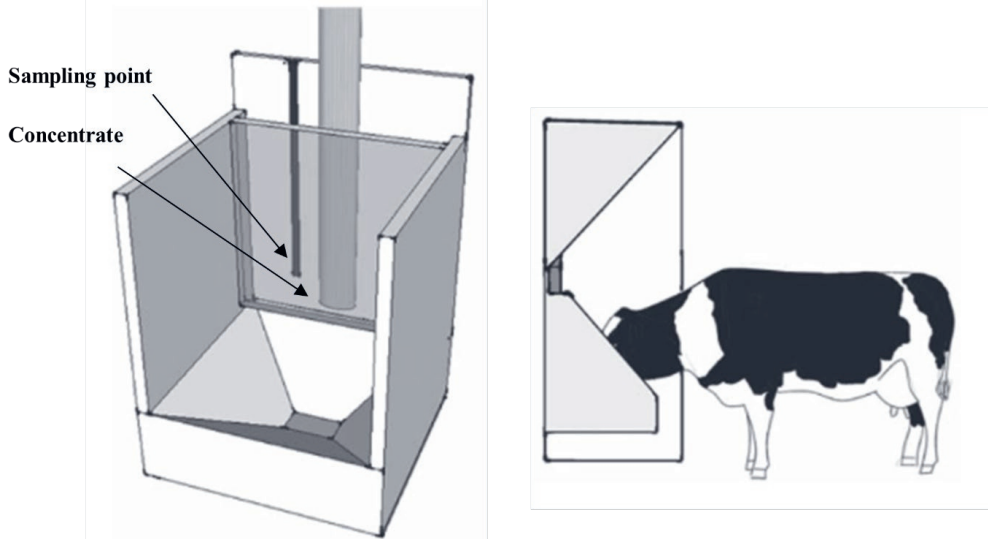

Figure 1.10. Schematic front (left) and side (right) view of a sniffer (Garnsworthy et al., 2012).

Open air monitoring device

Handheld laser methane detector

The handheld laser methane detector (**LMD**) is another approach to monitoring the methane concentrations of breath samples. With this technique, a portable device is held by an operator and taken either on a field or in a barn. Each animal must be approached (and monitored) sequentially by the operator. When sufficiently close to the animal (1 to 3 m), the LMD is oriented towards the head of the animal, and held to sample its exhaled air (Chagunda et al., 2009). The LMD uses infrared absorption spectroscopy to analyse the methane concentration of the collected sample. If animal movement allows it, data acquisition should last for a short (continuous) period of 2 to 4 min (Ricci et al., 2014). The data thus obtained represents the animal's respiratory cycles of the monitoring period. Only the detected peaks representing an increase in CH_4 resulting from an exhalation or an eructation are used in the data analysis (Ricci et al., 2014).

Studies reported that data obtained with LMD were in good, positive agreement with CRC when samples were measured simultaneously, *i.e.* LMD used in CRC (Chagunda and Yan, 2011; Sorg et al., 2017). However, sequential comparison of LMD and CRC estimates yielded mixed results, notably as a result of frequency and time of sampling (Ricci et al., 2014). It was shown that collected values must then be differentiated and segregated in two categories (exhalation and eructation) to improve its correlation with CRC measurements. The authors however reported it to be a difficult process. Additional difficulties linked to approaching unrestricted cows, and the effect of duration (Boré et al., 2022), relative humidity, pressure, wind speed and direction (Chagunda et al., 2013) on the LMD' methane measurements. However, the high portability of the device has the advantage of enabling spot sampling on varying practical conditions and production systems (Chagunda and Yan, 2011; D. et al., 2018).

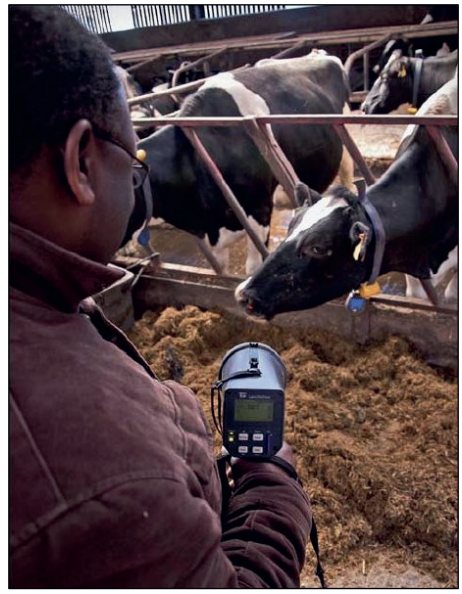

Figure 1.11. Picture of a handheld laser methane detector (Chagunda et al., 2013; Credit: A. Ross).

1.4. The challenges of practical monitoring

Monitoring enteric methane production in practical conditions excludes the use of CRC and inevitably comes with challenges. First of all, this type of devices generally either rely on the voluntary visit of animals into the system (*e.g.* GEM, sniffers, CHS), or requires the presence of an operator (*e,g.* LMD, PAC). In either case, only spot samples are possible, with minimal to inexistant control on the sampling scheme. Considering the general non-linearity of methane production after feed intake, converting snapshots into daily estimates is subject to errors. It has now been well established that the confidence level of a MPR estimate depends on the number of measurements taken each day for an animal (Cottle et al., 2015), their duration (Arthur et al., 2017), and their distribution over a complete 24-h period (Della-Rosa et al., 2021). In addition, feeding frequency diverge between individuals, which naturally eat varying quantities, and at different times, speeds and frequencies. These factor have a major impact on the number of daily samplings and their intervals that are necessary for spot sampling techniques to obtain reliable methane production estimates (Lee et al., 2022; van Lingen et al., 2023). However, sampling frequency cannot be easily altered, making levers for action limited. Models could potentially prove useful with this matter, for example if used to convert discrete measurements into daily rates by compensating for missing parts of the production curve. However, such

models have not yet been validated and their added value remains to be proven. The use of a tracer gas (*e.g.* SF6), for its part, allows for a continuous monitoring over multiple days, and is not restricted to indoor use. While this represents an advantage, this method requires cows to wear equipment and to be handled, which may cause stress. Additionally, SF_6 is a highly potent GHG with a GWP of 23500 (Myhre et al., 2013), which's use should be limited.

Secondly, additional challenges come with the collection of the sample itself. As about 98% of enteric methane is released by ruminants through exhalation and eructation (Johnson et al., 2000), breath samples should be collected and analysed. However, collecting breath air implies dealing with dilution, a problem that amplifies for devices operating in the more open environments (*e.g.* CHS, LMD). Indeed, the level of dilution of breath samples has been shown to be affected by two main factors: the airflows (speed, pattern, direction) around the device (Wu et al., 2016), the muzzle position and its distance to the inlet (Huhtanen et al., 2015a). Airflows can only partially be modified or avoided, for example by placing panels or curtains, and selecting the positioning of the device with care. Computational Fluid Dynamics (CFD) can be of help to model airflows in the livestock buildings (Rong et al., 2016), and to find the most optimal location to place the device. Head - and thus muzzle - positions are not to be altered, as the animals should be able to move freely. Nonetheless, the angle and distance between the muzzle and the inlet has been shown to be highly correlated with measured gas concentrations (Huhtanen et al., 2015a), therefore impacting the MPR estimate. The monitoring devices thus need to include sensors that monitor this distance so that monitoring ceases when the distance becomes too great (as with the GEM), or for the measurements to be filtered out at a later stage.

Additionally, background methane concentrations must be monitored in parallel to any device's enteric methane measurement. Background concentrations must be subtracted from the levels contained in the breath, so that methane produced by a cow of interest can be differentiated from the emissions of the barn (herd, manure). However, background methane concentrations vary in space and time (Wu et al., 2016), meaning that the location, timing, and frequency of background sampling can lead to monitoring different concentration levels. Errors made in background estimation will reflect on the accuracy of the MPR estimate. However, the high uncertainty that can be linked to background concentration measurements can be overcome when the concentration of the breath sampler is much greater than the background levels (McGinn et al., 2021). Aiming for the accumulation of breath air to increase its methane concentration can be one approach to take.

Finally, the levels of accuracy and robustness that are desired are dependent on each study and its objectives (*i.e.* the study of relative differences between animals or the monitoring of absolute levels of methane production). Devices designed to monitor enteric methane in practical conditions must therefore be conceived and tested in accordance with what they wish to evaluate.

1.5. Study aims

The lack of reliable practical devices to monitor enteric methane production at large scale is a hindrance to the evaluation and implementation of GHG reduction strategies in livestock production. Although many devices have been developed for this purpose, they do not yet operate satisfactorily (Hill et al., 2016), and many challenges remain to be tackled. This thesis aims at addressing some of these challenges.

The framework of this study revolves around the exploration of three key aspects. Firstly, it aims to investigate the accuracy of methane production rates estimated by two practical devices, both in the presence and absence of animals. Secondly, it explores the conversion of discrete measurements (resulting from spot sampling methods) into daily production rates, taking into account the general non-linearity of the postprandial methane production curve. Lastly, it seeks to identify the factors affecting and limiting individual measurements of enteric methane fluxes under practical conditions. This comes down to the following study aims:

- Develop an improved version of the cubicle hood sampler and test the performance of its embedded sensors.
- Investigate the ability of two practical devices (portable accumulation chamber and cubicle hood sampler) to assess methane production rates in sheep and cows respectively.
- Develop a model allowing to assess postprandial methane production at population and individual levels, and to convert discrete measurements into daily production rates.
- Report and discuss the challenges and limitations encountered when monitoring enteric methane in ruminants under practical conditions, and provide perspectives.

1.6. Thesis layout

In **Chapter 2**, a validation study was conducted with a set of 10 portable accumulation chambers to investigate their ability to measure absolute methane production rates and to rank animals according to these levels. This was achieved through the completion of recovery tests using a mass flow controller, which releases known masses of methane. Chambers were tested for their ability to repeatedly record the same mass, as well as for their capacity to accurately monitor a range of masses. This work represents the first real calibration test conducted in portable accumulation chambers, and proposes an accessible protocol to detect leakages and calibrate portable accumulation chambers' measurements.

Chapter 3 presents a first of its kind methane production model. In this chapter, we present an extension of the model of Crompton et al. (2011), which we reformulated into a Bayesian hierarchical kinetic model. In this so-called hierarchical methane rate model, parameters representing the shape of the methane production curve were pooled across cows (population-level), while the scale parameter was allowed to vary between cows. This allows the borrowing of information across cows, while representing between cows variability. Fitting the model with climate respiration chamber data showed how the model can be used to convert discrete measurements into non-linear curves, before deducting daily methane production rates.

In **Chapter 4**, the latest prototype of the cubicle hood sampler is presented. This device aims at monitoring the daily methane production of individual cows under barn conditions and in a non-intrusive manner. Its design (components, dimensions, settings) are presented. Preliminary results, such as the recovery rates of the four units, and the performance of the cow identification and head monitoring systems are shown and discussed.

In **Chapter 5**, the ability of the cubicle hood sampler to estimate individual methane production rates and rank cows accordingly was investigated. This chapter details the study in which the methane production of twenty eight lactating dairy cows were estimated by climate respiration chambers and cubicle hood samplers. Direct comparison of these levels were done, before investigating the added value of fitting the hierarchical methane rate model with cubicle hood sampler data. This chapter reports the latest findings on the ability of the cubicle hood sampler to monitor methane production rates, and the challenges that arose from this study.

The last section, **Chapter 6**, connects all the findings collected in this thesis. The added value of each section is discussed, together with the limitations that have been encountered. The key

elements that affect the accuracy of the estimations of (individual) enteric methane production made under practical conditions are identified. Their interactions are highlighted, and propositions are made to resolve or better understand them. Recommendations and points of attention for further research are synthetized.

Chapter 2.

Monitoring enteric methane production in small ruminants with portable accumulation chambers

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Highlights

- Portable accumulation chambers are well suited to rank small ruminants according to their methane production rates.
- If uncalibrated, portable accumulation chambers are not suited to record absolute methane production levels.
- Recovery tests are an effective way to assess the accuracy of devices monitoring gaseous exchanges freely of extraneous factors.

Abstract

Using portable accumulation chambers (PAC) is an attractive approach to recording methane (CH_4) production of small ruminants. Mass flow controllers (MFC), for their part, are an effective way of validating PAC measurements, as they allow to simulate methane production rates freely of extraneous factors. The present study describes a series of tests carried out to evaluate the accuracy and precision of methane mass recordings of eight PAC against known CH_4 masses released by a MFC. Across the tested range, the PAC were able to recover between 67.6 and 74.5% of the true CH_4 released. No significant differences were detected between the different PAC, but a statistically significant linear shift was detected over the mass range. Therefore, PAC as currently used are not well suited for applications looking at absolute production levels requiring high absolute accuracy. If recovery tests are conducted regularly across the measurement range, a calibration factor could be generated to correct for the inaccuracy. These PAC were however well suited for investigating relative differences between animals, *e.g.* ranking animals to compare differences in CH_4 production between breeds. The methane recordings made by these chambers were highly precise, with low coefficients of variation between replicates (0.0-2.4%) and high repeatability (>0.99). Furthermore, the correlation between released and recorded CH_4 masses was very strong and positive ($R>0.99$). Overall, the mass recovery test presented here provides a feasible method for harmonizing methane monitoring procedures using PAC between research groups, thereby improving joint efforts aimed at mitigating greenhouse gas production in small ruminants.

2.1. Introduction

Over the past decades, the potent greenhouse gases (GHG) emissions from livestock farming have kept rising. In 2019, enteric methane from ruminants (resulting from the enteric fermentation process) accounted for 44.3% of the global sector's emissions (FAO, 2021b). With a global warming potential 28 times that of carbon dioxide (CO_2) (IPCC, 2021) and a conversion to the long lived pollutant CO_2 (IPCC, 2014) after reaching its lifetime of 12 years (G. Myhre et al., 2013), its emission must imperatively be mitigated.

Small ruminants such as sheep and goats make up over 50% of the global ruminant production with a world population exceeding two billion animals (Gilbert et al., 2012). Sheep and goats are raised for fibre, meat, milk and hides over a wide range of climatic conditions and production systems, particularly in marginal areas and extensive systems where conventional crop production is infeasible (Alberto et al., 2018; Hristov et al., 2013a). Reducing the CH_4 emission from sheep and goats is therefore high on the international research agenda, with strategies spanning multiple disciplines such as animal breeding, nutrition, and microbiology (Hess et al., 2022; Jonker et al., 2018). All disciplines require accurate and precise measurements from which to gauge the response and efficacy of different mitigation strategies.

However, different scopes of applications may require different levels of accuracy and precision in methane mass estimation. For instance, strategies looking at relative differences between individuals or groups of animals, such as ranking animals for breeding or comparisons between breeds, may forego some accuracy in place of precision, correlation and ease of measurement (Lassen and Difford, 2020). Conversely, strategies which concern absolute differences between treatments require high levels of measurement accuracy and precision.

Climate respiration chambers (CRC) are widely regarded as the "gold standard" method for this type of measurements. In this system, animals are confined for several days in individual open-circuit compartments in which metabolic and gaseous exchanges are monitored. Inlet and outlet airflows as well as the internal climate conditions (temperature, humidity) are fully controllable. Monitoring of the concentration difference in the inlet and outlet gas mixtures, coupled with flow information, allows for CO_2, O_2, and CH_4 fluxes to be calculated (Kuhla et al., 2015). However, this methodology is expensive, labour intensive and has lower throughput, which can prove prohibitive to measure large numbers of animals under on-farm settings (Garnsworthy et al., 2019; Robinson et al., 2020). Furthermore, the prolonged confinement within the chamber

restricts natural behaviours and induces social isolation which could result in behavioural changes, and reduced feed intake (Gastelen et al., 2015).

A low cost and relatively higher throughput alternative is the portable accumulation chamber (PAC) (Goopy et al., 2011), where an animal is confined into a closed chamber for a period of 40 to 120 min (usually 50 min) and the changes in gaseous concentrations are recorded (Jonker et al., 2020b). The volume of the chamber, corrected for the estimated volume of the animal, is then used to estimate the mass flux of (methane) gas produced by the animal. The highly portable nature of the PAC has enabled researchers across Australia, Ireland, New Zealand, Norway, and Uruguay to record CH_4 production from thousands of sheep, primarily for ranking of animals and selection experiments (Jonker et al., 2020b). However, there is a large variation in design of PAC across research groups and limited consensus on best practices (Jonker et al., 2020b) which may lead to unequal performances and, therefore, incomparable results.

Despite their extensive use across the globe, the absolute accuracy and precision of the PAC' measurements, and the ability of this system to rank animals based on their methane production rates (MPR) have received little attention. The majority of research has involved subsequentially recording sheep in CRC and in PAC to evaluate the level of correlation between the MPR levels estimated by both methods. For example, Goopy et al. (2011) found the methane estimates of PAC and CRC to have a correlation of 0.71, while Robinson et al (2015) reported correlations between 0.0 and 0.19 when adjusted for liveweight and feed intake. For logistical reasons, sheep cannot be recorded by both methods simultaneously, which poses a challenge to researchers to determine if differences between measurements with both methods are due to true differences in the methodologies or as a result of extraneous physiological factors, such as intra or inter-day(s) variations, feed intake or dietary changes between measurements (Jonker et al., 2020b; O'Connor et al., 2021a, 2021b). To allow for a validation of the PAC's ability to accurately monitor individual MPR from sheep and use these results to rank individuals, it is essential that the impact of biological and methodological factors on accuracy are distinguished. To achieve this, a thorough validation of each used PAC must be conducted. Therefore, the objectives of the present study were to determine the absolute measurement accuracy, precision, and ranking ability of the different chambers, and across the range of MPR produced by sheep. To achieve this, we conducted an intra-laboratory ring and a range test, in which precise and known fluxes of methane were released into the PAC units before calculating the recovery rates achieved by the system. This can elucidate the absolute measurement accuracy gap between PAC, as well as the precision and efficacy of ranking across the range of MPR at which the

PAC are expected to perform, which can range from 0.25 to more than 2 g h^{-1} depending on ration, postprandial duration, breed, age, and physiological status (Jakobsen et al., 2022; McHugh et al., 2022; Navajas et al., 2022).

2.2. Material and Methods

2.2.1 Portable accumulation chambers

The ten PAC used in the present study have been mounted in a truck, allowing transportation of the equipment to commercial farms and remote pastures. The design of the truck was adapted to allow for heating when conditions were extremely cold, thus preventing the impact of the low temperature and high moisture on the gas measurements. Ten identical chambers were built-in, with five on each lateral side of the truck separated by a walkway. Each chamber measured 113 x 84 x 115 cm (L x W x H, Figure 2.1) which corresponds to a total internal volume of 1127 L once corrected for internal parts. The chambers' outer structure was made of welded aluminium sheeting and made hermetic by a pneumatic rubber lining around the door opening. These doors were equipped with an inlaid polycarbonate panel to allow visual observation of the sheep and reduce isolation-induced stress by allowing animals to see each other. A gas extraction system (Figure 2.1, *E*) was fitted to each row of PAC on either side of the truck, and allowed for the removal of residual gases from previous measurements before carrying out a new one. All PAC could be flushed individually or simultaneously as they were individually connected to the exhaust system by a supple PVC piping equipped with a valve (*V*). At the time of use, chambers were 2 years old, and had been used to record methane production from more than 4500 Norwegian white sheep.

Each PAC was equipped with one pressure manometer (GM511, Benetech, Shenzhen, China) measuring the absolute pressure difference with the ambient air, and placed on the upper panel of the chamber (*M*) and providing a reading of the internal chamber pressure every 0.5 sec. This information allowed the operators make sure that the chamber was air tight. An Eagle 2 multi gas analyzer (*E2*, RKI Instruments, Union City, USA) was connected at will to any of the chambers with a probe fitted through a compression port (*CP*). The Eagle 2 detection principle relies on infrared spectroscopy and thermal conductivity, and permits measurements of methane, oxygen, and carbon dioxide gas concentration with one reading every 15 sec for each gas. For methane, reading increments of the Eagle 2 were of respectively 5 ppm in the 0-200 ppm range, 10 ppm in the 200-1000 ppm one, and 50 ppm in the 1000-10000 ppm scale.

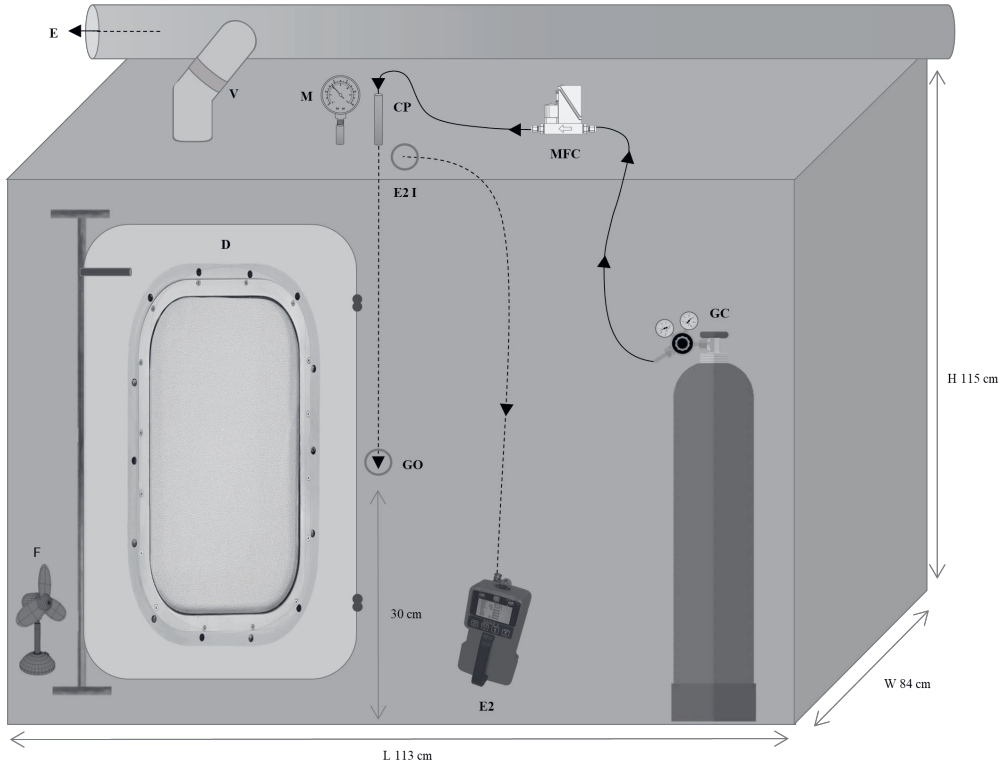

Figure 2.1. Schematic view of the portable accumulation chamber. From top to bottom: E: Exhaust duct, V: Valve (connecting the chamber to the exhaust/flushing system); M: Manometer; CP: Compression Port; MFC: Mass Flow Controller; E2 I: Eagle 2 Inlet; D: Door (equipped with a window); GC: Gas Cylinder (methane); GO: Gas Outlet; F: Fan; E2: Eagle 2 gas analyser. Fan and Eagle 2 are placed inside the chamber while the other components are placed outside. Gas tubes and flows are represented by dashed line when located inside, and plain lines when located outside the chamber.

2.2.2 Calibration gas and mass flow controller

All the tests subsequently described were carried out using a mass flow controller (MFC) releasing known fluxes of methane in ranges between 0 and 500 $ml_n\ min^{-1}$ at an accuracy of ±0.5% reading deviation plus ±0.1% full scale (EL-FLOW Select F-201-CV-500, Bronkhorst High-Tech B.V., Ruurlo, The Netherlands). The MFC was calibrated prior to the experiments at the Air Quality Lab of Wageningen University & Research (Wageningen, The Netherlands) with a gas mixture of methane (3000 ppm) in nitrogen. Calibration was done against two

reference flow meters: one Defender 510L (range 0-500 ml) and one Defender 510M (range 0-5000 ml) (MesaLabs, Lakewood, USA), themselves most recently calibrated in, respectively, December 2020 and December 2021 by the third-party TPF control B.V. (Boven-Leeuwen, The Netherlands). The calibration line [eq. 2.1] was obtained for the MFC for the range of 0-500 $ml_n\ min^{-1}$:

$$\text{Measured flow lab} = 1.36 \times \text{Set flow lab} + 1.20 \qquad \text{[eq. 2.1]}$$

where Measured flow lab is in $ml.min^{-1}$ and Set flow lab in $ml_n.min^{-1}$.

The desired injection rates were calculated depending on each experiment. To modulate injection rates, the MFC relied on the thermal bypass measuring principle and the controllable opening of an integrated valve coupled to a laminar flow element (stack of stainless-steel discs with precision-etched flow channels). Prior to use, a minimum warm-up time of 30 min was required for the internal components of the MFC to reach the temperature required for accurate injection rates. Connections between MFC, inlet and outlet were made with polyethylene tubing ($\varnothing_{internal}$ = 4.35 mm). The calibration gas used during the leakage test was CH_4 (2000 ppm) in nitrogen, and an ultra-high purity methane one (≥ 99.9995 purity methane) for the intra-laboratory ring and range tests (Linde Gas AS, Oslo, Norway).

2.2.3 Leakage test

A leakage test was conducted on each of the 10 PAC to ensure that they were all pneumatically sealed. After sealing of the door, the methane gas mixture was released inside the chamber at a flow of 681 ml min^{-1} until a pressure of 2 mbar was reached (manometers M, Figure 2.1). Once this pressure threshold was reached, the injection ceased and the manometer pressure reading was monitored every minute for 10 min. From this test, one chamber was found to have a severe leak which did not allow the chamber to reach the pressure threshold, and a second chamber lost gas pressure within 10 min after reaching the threshold. Both chambers were excluded from the ring and range tests as they could not be repaired soon enough. When repaired (later stage), both leakages appeared to be due to cracks in the rubber joints that normally make the doors airtight. The other eights PAC were found to be hermetic and included in the study.

2.2.4 Ring test

The ring test was conducted to investigate the inter-chamber measurement repeatability. For this, 1.59 g of methane were injected in each chamber in (consecutive) triplicates using the MFC. As described in Figure 2.1, methane was injected through the compression port of the chamber (*CP*). Gas outlet (*GO*) was suspended at approximately 65 cm from the floor of the PAC to mimic the head position of a standing sheep. A battery-operated mini-fan (*F*, Clas Ohlson, Insjöm, Sweden) was placed on the PAC's floor during the measurements to simulate the natural mixing of gases that would normally have occurred from sheep movements, body heat, and respiration. The MFC was set at an injection rate of 681 ml min^{-1}. Accounting for a mass of 0.72 g CH_4 per L of a 999999 ppm calibration gas, the duration of injection was calculated as:

$$t = \frac{T_m}{0.00072 \frac{gCH_4}{L} \times IR} \quad \text{[eq. 2.2]}$$

where T_m is the target mass in g, IR the injection rate in ml min^{-1}, and t the time in min.

This protocol was designed to favour the number of replicates per PAC unit tested, which provides essential information on repeatability and precision. Therefore, we opted for the quickest injection duration that allowed to reach the target mass, unlike the classical 40 to 60 min measurements normally conducted with live animals. As a result, a total of 1.59 grams of methane were injected in each chamber after 195 sec of injection [eq. 2.2], at which point the gas valve was closed.

Methane concentration was measured by one Eagle 2 (*E2*) which was directly placed into the PAC as the only *CP* was used by the injection line. The device was positioned on the floor while its sampling probe (L = 1.5 m) was attached to the ceiling of the chamber (*E2 I*) to mimic its regular positioning. The background methane concentrations (ppm) (residual gases present in the chambers) were recorded at t_0 and subtracted from subsequent readings. One concentration reading was recorded when injection stopped (t_{195sec}), and then once every minute for 3 min. From this, an average value of the methane concentration was computed over the 3 min post-injection, completed by its standard deviation, which provided insights on the level of stability of the measured concentrations and resulting recovery rates. After that, the PAC was flushed through the exhaust (*E*) system to remove the residual gas. This process was repeated until all PAC had been tested in triplicates. The methane concentrations monitored by the Eagle

were then used to calculate the estimated volume of methane (L) present in the chamber using the equation of Jonker et al. (2020b):

$$V_{CH_4} = \frac{[CH_{4_{PAC}}] - [CH_{4_B}]}{1000000} \times V_{PAC} \quad \text{[eq. 2.3]}$$

where V_{CH_4} is the volume of methane (L), $[CH_{4_{PAC}}]$ is the stabilized methane concentration (ppm) monitored over the final 3 min, $[CH_{4_B}]$ is the background methane concentration (ppm) at t_0 , and V_{PAC} the internal volume of the PAC (L).

The injected volume was then converted into mass using the ideal gas law principle:

$$m_{CH_4} = V_{CH_4} \times STPD \times \frac{M_{CH_4}}{M_V} \quad \text{[eq. 2.4]}$$

where m_{CH_4} is the methane mass (g), V_{CH_4} the injected volume (L), M_{CH_4} the molar mass of methane (g); and M_V the molar volume (L) of methane at STPD (standard temperature and pressure dry).

The STPD correcting factor was calculated as indicated by Alferink et al. (2015).

2.2.5 Range test

The range test was conducted to assess the absolute measurement accuracy of the setup across the range of methane fluxes which are expected to be measured in practice. Two chambers were randomly selected and tested in (consecutive) duplicates across the range of 0.5 to 2.5 g. This range was established to correspond to the diversity of MPR normally monitored for the type of sheep with which this system is used.

Methane masses of 0.48, 1.08, 2.04 and 2.52 g were injected in the two chambers over 60, 135, 255 and 315 sec respectively. For each treatment, the duration of injection was calculated using formula [eq. 2.2]. The protocol followed the general procedure established for the ring test, namely: a flushing of the PAC prior to each measurement followed by the injection of methane gas into the chamber using the MFC at an injection rate of 669 ml min^{-1}. Methane concentrations in the PAC were recorded with an Eagle 2 at t_0 (background concentration), at the cease of injection, and once every minute for 3 consecutive minutes. The average concentration values obtained over the 3 min post-injection were used for further analysis.

2.2.6 Statistical Analysis

All statistical analysis were done with R x64 4.1.0.

Recovery rates were calculated according to equation [eq. 2.5]. They provided an indication on the percentage of methane mass PAC were able to recover, as well as information on the presence of systematic errors between injected and recovered values.

$$\text{Recovery rate} = \frac{m_{\text{recovered}}}{m_{\text{injected}}} \times 100 \qquad \text{[eq. 2.5]}$$

The coefficient of variation (CV) was computed for each PAC unit to determine the magnitude of the variation between replicates. A linear mixed model was fitted on the recovery rates across the range test to assess the proportion of variation explained by differences in the range relative to the replicate variation. This was done using the Sommer package (Covarrubias-Pazaran, 2016), which gave the following form:

$$y_{ijk} = \mu + PAC_i + Range_j + e_{ijk} \qquad \text{[eq. 2.6]}$$

Where, y_{ijk} is the recovered mass of CH_4, of the i^{th} PAC chamber (i = 2 levels), of the j^{th} range setting $Range_j$ (j = 5 levels correspond to 0.5 g to 2.5g CH_4 in 0.5g increments). The random effect $Range_j$ is assumed to be normally distributed with a mean of zero and variance structure $\sim ND(0, I\sigma^2_{Range})$. Similarly, the residual error term of the k^{th} replicate of the j^{th} range of the i^{th} PAC was assumed to be $\sim ND(0, I\sigma^2_e)$. The repeatability expressed as a coefficient was computed according to Wolak et al. (2012) as:

$$Rep = \sigma^2_{Range} / \left(\sigma^2_{Range} + \sigma^2_e\right) \qquad \text{[eq. 2.7]}$$

A linear regression of the masses of methane injected by the MFC on the methane recovered by the PAC was performed to test for any potential bias in recovered CH_4 values. It was used to calculate R^2, which indicates the percentage of variance in the response variable that is explained by the regression model. Pearson's correlation coefficient (r) was derived from R^2, and denotes the level of correlation between the injected and recovered methane masses. In addition, the Root Mean Square Errors (**RMSE**) were calculated to estimate the measurement errors made by the device, with smaller values reflecting a lower level of error. One-way ANOVA were used to detect potential presence of significant differences in recovery rates between PAC, and between injected masses.

2.3. Results

2.3.1. Ring test

The PAC in the current study had fairly consistent and systematic recovery rates of approximately 69.4% ±1.1 (Table 2.1, Figure 2.2). In addition, there was a low average coefficient of variation (1.6%) across replicates and a substantial repeatability of 0.99. The computation of the one-way ANOVA yielded a p-value of 0.207, showing that there were no significant differences in the average recovery rates measured by the different PAC. An overview of the dataset can be found in Appendix A, showing notably the consistency in recovery rate observed over the 3 min post injection.

Table 2.1 Ring test (1.6g CH_4): Mean recovered methane mass (g) per portable accumulation chamber and standard deviation (three repetitions per chambers). Corresponding recovery rates (%), standard deviation in recovery rate between repetitions (%), and coefficient of variation across chambers (%).

PAC number	Mean recovered mass ± standard deviation, g	Mean recovery rate and standard deviation, %	Coefficient of variation in recovery rate, %
2	1.09 ± 0.007	69.63 ± 0.48	0.69
3	1.10 ± 0.020	69.24 ± 0.54	0.78
4	1.08 ± 0.043	68.42 ± 1.61	2.36
5	1.10 ± 0.027	69.47 ± 1.91	2.74
6	1.09 ± 0.013	67.84 ± 0.41	0.60
7	1.09 ± 0.010	69.83 ± 0.63	0.90
8	1.13 ± 0.027	70.44 ± 0.71	1.01
10	1.09 ± 0.006	69.59 ± 0.36	0.53
Grand mean	1.10 ± 0.024	69.37 ± 1.10	1.59

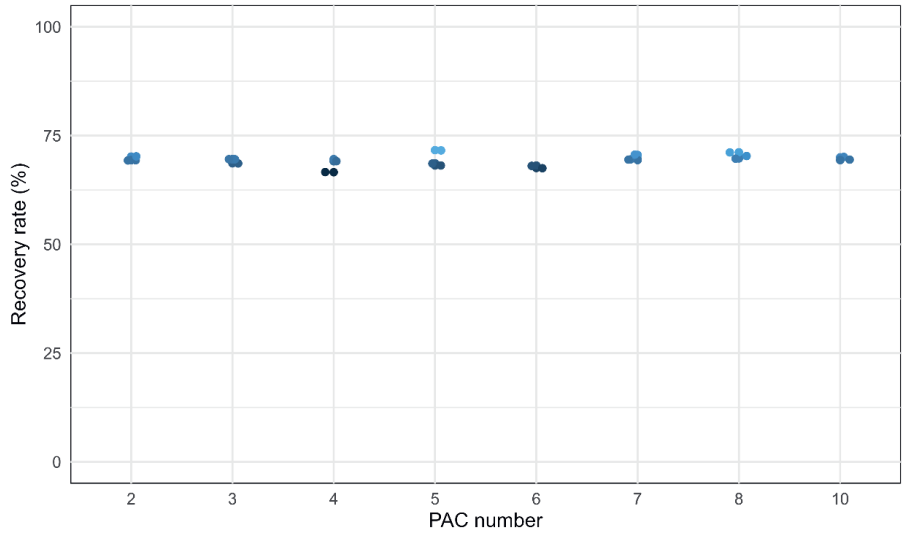

Figure 2.2. Recovery rates (%) obtained in the ring test during which 1.59g of methane were injected in triplicates (in duplicate for PAC 6) in the eight chambers that were retained after the leakage test. Scale of recovery rates going from lighter (higher values) to darker (lower values) blue.

2.3.2. Range test

Across the range of methane masses (0.47 - 2.48 g, Table 2.2), the two PAC had similar recovery rates, with an average of 70.6% ±2.1 and a low CV between replicates (2.9%). The linear relationship between injected and recovered methane masses appeared to be strong and positive ($R^2 = 0.999$, Figure 2.3) and RMSE to be low (0.515). However, there was a significant shift in recovery rates with increasing masses (p-value < 4.88e-5, Figure 2.4). An overview of the dataset can be found in Appendix B, showing notably the consistency in recovery rate observed over the 3 min post injection.

Table 2.2. Range test (treatments 0.5 to 2.5g CH4): Mean and standard deviation of the mass (g) recovered by the two chambers for each of the five treatments levels. Average recovery rates (%) calculated from equation [eq. 2.5] and their standard deviation.

Mean injected mass, g	Mean recovered mass, g	Standard deviation recovered mass, g	Mean recovery rate and standard deviation, %	Coefficient of variation in recovery rate, %
0.47	0.35	0.005	73.88 ± 1.02	1.39
1.06	0.77	0.000	71.48 ± 0.00	0.00
1.54	1.09	0.021	68.82 ± 1.63	2.37
2.01	1.43	0.000	70.01 ± 0.06	0.09
2.48	1.74	0.008	69.18 ± 0.33	0.48

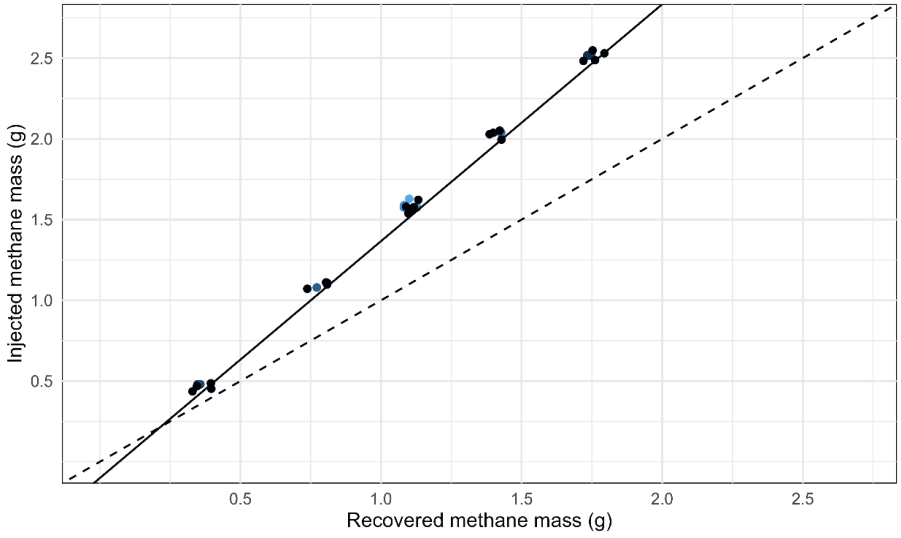

Figure 2.3. Linear regression of the five methane mass injected by the MFC during the range test against the mass recovered by the two PAC (regression line: x = -0.039 + 1.47y). The regression line x = y is represented by the dashed line. The scale of distances between values and regression line are represented by shades of blue, going from lighter (higher values) to darker (lower values) blue.

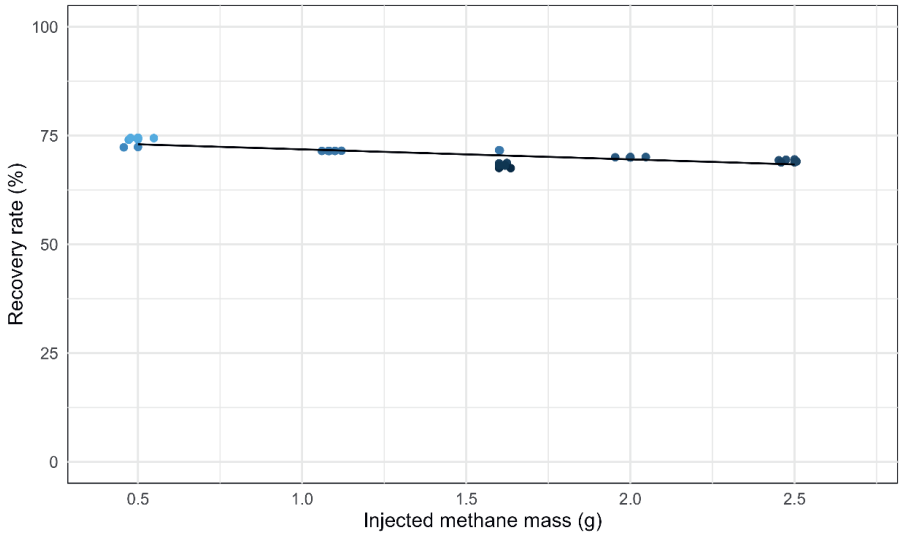

Figure 2.4. Recovery rates (%) obtained in the range test during which methane mass of 0.5 to 2.5 g were injected in the two chambers that were randomly selected for the test (regression line: y = -2.24x + 74). Scale of recovery rates going from lighter (higher values) to darker (lower values).

2.4. Discussion

Portable accumulation chambers have traditionally been used for animal ranking experiments (Jonker et al., 2018), or to compare relative differences between experimental groups, such as breeds or lineages. For this type of research, absolute measurement accuracy is not strictly required, and instead relative differences between measurements are of interest (Fredeen, 1986).

Adequate precision of the measurements as well as correlation to the true levels are therefore of primary importance (Lassen and Difford, 2020). However, there is increasing interest in expanding their use to different scopes of applications, for instance GHG inventories or as a response variable in dose-response trials used for investigating physiological response and nutrition effect, where the absolute accuracy is of importance (Jonker et al., 2020a). Here, we interpret findings of the ring and range tests, firstly in terms of accuracy to evaluate the potential use of PAC in these applications, and secondly in terms of correlation and precision to validate their use in ranking experiments.

2.4.1. Accuracy of the PAC methane measurements

The recovery rates across the ring test revealed that the PAC are consistently under recording methane mass by approximately 30% of the true value. There were no significant differences between the chambers, demonstrating the systematic nature of this under recording. The ring test revealed that the recovery rates have a small but significant linear shift in accuracy across the measured range, going from 73.88 ± 1.02 to 69.18 ± 0.33 between the 0.5g and the 2.5g injections (Table 2.2, Figure 2.3). This shows that it is necessary to conduct recovery tests across the desired measurement range so that shifts in accuracy can be corrected for, without which PAC cannot be used as a methane measurement device in studies requiring high measurement accuracy. Mass recovery testing in PAC yet remains rare, which makes comparisons to literature challenging. Most studies either do not conduct recovery tests, or solely perform leakage tests which might be confused with recovery tests. For instance, Goopy et al. (2011) released SF_6 tracer gas into PAC and monitored the inner SF_6 concentrations of samples taken 2-h after injection of the tracer gas. Measured concentrations were found to be ranging between 98 and 99% of the concentration directly after injection. Similarly, Paganoni et al. (2017) released CH_4 gas up to a fixed initial concentration and recorded the concentration in the PAC over a 2-h period. In their case, concentrations were found to equate to 95% of the original value. However, both approaches do not constitute true recovery tests, as the true mass of injected gases were not known. In other words, even though the volume of the chamber and measured gas concentrations are (near) constant over a period, the mass estimated may still be inaccurate if for example the constant measured gas concentration readings are themselves inaccurate. These inaccuracies will remain undetected in concentration tests without knowledge of the true injected mass and thus the true gas concentration. These studies thus provide limited information of the ability of PAC to accurately monitor the mass of specific gases but are a good indicator of potential leakage.

Previous comparisons of sheep and lambs' MPR as (sequentially) measured by PAC and CRC are another approach towards testing the accuracy of PAC measurements. Overall, these studies have indicated considerable under recording of CH_4 production by PAC. For instance, Goopy et al. (2011) recorded the methane production of 39 sheep in CRC for 22-h, and subsequently in PACs for 1-h. The daily MPR as estimated by the PAC was only 23.2% of the 29.8 g d^{-1} monitored by the CRC. In a larger scale trial, Jonker et al. (2018) recorded daily CH_4 production in 3601 lambs in CRC and in PAC, with the latter recording on average 7.5 g d^{-1} as opposed to the 24 g d^{-1} in the CRC. These results corresponded to a recovery rate of approximately 31%.

However, it must be noted that these studies were not aiming at investigation the recovery rates of the chambers used and their designs were focussed on ranking of sheep. In addition, the differences between measurements may most certainly have been influenced by the diverse sampling days, times (postprandial duration) and diets. Thus, this approach has limited use as differences in recovery rates and accuracy due to methodological factors may be confounded with differences due to biological factors. However, they do give an indication of the range of recovery to be expected under challenging scenarios. Assessing the recovery of sets of PAC should therefore be done in the absence of extraneous factors before conducting any study with live animals, as the approach described in this study allows. This is crucial for applications requiring absolute accuracy of gas measurements. In the case of ranking experiments, it is not necessary to determine whether there are systematic differences in accuracy or differences between PAC, provided that potential differences between chambers are appropriately accounted for in the statistical analysis conducted (for example by including a chamber effect). However, it remains best practice to monitor differences in recovery rates and to correct these mechanically rather than statistically where possible. An important consideration for the use of PAC in applications requiring high accuracy is the realization of a calibration procedure against a reference method. The approach taken for the range test in this study can be used for this purpose. This requires making use of the regression equation shown in Figure 2.3 to align PAC measurements with the true value. However, this calibration standard is likely to be only relevant for the conditions under which it was performed, meaning that complementary routine recovery tests should be conducted. This has become common practice with the gold standard method CRC, and is best exemplified by a study conducting a ring test of twenty-two CRC at six different facilities in the United Kingdom (Gardiner et al., 2015). Recovery rates were found to vary across CRC, ranging from 59 to 115%, demonstrating both positive and negative shifts in accuracy, and showing very clearly that even the gold standard methods can differ in absolute accuracy. The authors thus highlighted the importance of recovery testing, in terms of both accuracy and transparency. As a result, and after correction of the measurements values using the recovery rate as a calibration factor, the combined uncertainty across chambers was reduced from 25.7% to 2.1%. This has led to international consortiums calling for the publishing of recovery rates as a prerequisite to publishing gaseous exchange data and as an approach to harmonize measurements both for respiration chambers and other methods (Gerrits et al., 2017; Hammond et al., 2016a).

Another approach is to identify the cause of the shift in accuracy. Depending on the monitoring device, this can be a tedious approach. However, in static chambers, such shifts can only be the result of a leaking unit, errors of injected mass, or a shift in the measurement accuracy of the sensor. As the chambers were tested for leakage, and the MFC was calibrated against two reference flowmeters, only the gas analyser remains as potential source of error. Offsets (linear or not) are common in analysers and simply require to be calibrated thorough the measurement range at which they are expected to operate. These offsets can lead to varying levels or under or overestimation of the measured concentrations, leading to shifts in recovery such as the one observed here. In addition, humidity has been shown to be an inference factor of infrared sensors, being correlated to the gas concentrations that are measured with this technology (Dinh et al., 2016; Singh and Malarvili, 2020; Yuliang et al., 2017). Gas analysers using infrared spectroscopy (alike the Eagle 2) should therefore be calibrated across the concentration and the humidity range at which they are expected to operate, allowing these offsets to be calibrated for.

Although the instrument was auto calibrated, the instrument's "autocalibration" function constitutes a zero point calibration and not a full span calibration. This does not allow to detect potential shifts in accuracy that usually occur across the measurement range, and does not account for humidity levels. Subsequently, we determined that a span calibration is possible and necessary for this sensor, but requires sending the analyser out of the country, which was not possible then. Optimally, the current gas analyser should be calibrated with methane gas concentrations ranging between 400 and 2300 ppm to operate over the whole range of expected CH_4 produced by sheep. We expect that applying such a calibration to the dataset will most likely increase recovery rates and correct for the shift in recovery, therefore probably leading to a higher measurement accuracy. A full sensor evaluation is needed to determine if a span calibration will increase the recovery rates to 100% or if systematic sensor inaccuracy is persistent and to what extent this affects recovery. For instance, a number of methane sensor comparisons have been conducted and found systematic and significant differences between different sensors, indicating that depending on the sensor type, and regardless of any other factors, recovery rates will differ due to sensor type alone (Difford et al., 2016; Rey et al., 2019; Sypniewski et al., 2019).

2.4.2. Precision and ranking of PAC methane measurements

Precision reflects the amount of random or non-systematic errors that can afflict measurements, and is particularly important in ranking applications as it can cause unwanted re-ranking and bias correlation estimates downwards (Lassen and Difford, 2020). It is prudent to first evaluate a system for these non-systematic errors prior to the additional of live animals which introduce their own set of random biological variation. The repeatability in the absence of animals gives an indication of the precision of the technical aspects of a system, if this is low researchers may wish to find way of improving the measurement system prior to live animal experimentation. The ring test revealed substantial precision of measurements with a repeatability close to unity (0.99) and very low CV ranging from 0.0 to 2.7% across both ring and range tests, together demonstrating very low variance between replicates. These findings are encouraging and offer a theoretical upper limit to what precision can be achieved with PAC systems with-out random biological variation introduced by live animal measurements. In addition, the strong and positive relationship between injected and recovered methane masses ($R^2 = 0.999$, Figure 2.3) and low RMSE (0.515) show that the tested PAC can differentiate methane production levels, even in spite of the presence of systematic errors. The chambers tested here are therefore well suited to rank animals as they can record relative differences in the tested range.

By using a MFC, we have removed the random extraneous confounding biological factors introduced when monitoring live animals. Direct comparison with research works in which MPR were sequentially measured by PAC and CRC is weakly informative with regard to the accuracy of the measurement system, as it is usually not the purpose of those studies (aiming at investigating the precision of the relationship between both methods). However, it does show how these results can vary in the presence of biological factors, and emphasizes the importance of recovery tests free of animals. For instance, O'Connor et al. (2021b) recorded 48 ewes lambs over 17 consecutive days in PAC and found the repeatability between measurements to be moderate (0.36) and with large variations between days. Goopy et al. (2011) recorded MPR in 39 sheep (following the same diet) with CRC for 22-h, followed by a single 1-h recording in the PAC. They found positive Pearson's correlations ranging from 0.69 to 0.71. As for their part, Jonker et al (2018) recorded methane in 3601 lambs with CRC and PAC and found lower phenotypic correlations between methods ranging from 0.004 to 0.27, however their design was aimed at genetic correlations which were higher ranging from 0.41 to 0.67. Lastly, Robinson et al. (2020) recorded 510 ewes that were on the same diets with PAC and CRC, but 4-d or more

apart, and found phenotypic correlations of 0.63 - 0.64. These studies suffered from the unavoidable non-simultaneity of the CRC and PAC measurements on live animals.

2.4.3. Practical implications

Depending on the scope of applications, researchers may require different degrees of accuracy, precision, and correlation from a system monitoring MPR. Previous studies have approached method evaluation by non-simultaneously recording the MPR of live animals in PAC and CRC. Methane production rate levels were found to vary between methods, effectively confounding differences induced by biological factors and varying measurement accuracy. In the current study, we outlined an approach that uses precise and known methane mass release, and therefore enables researchers to test all three facets of measurement agreement free of animal variation.

We demonstrated that the precision of repeated measurements of the tested PAC (repeatability = 0.999) and the correlation to the true methane mass were equally high (correlation = 0.999). It confirmed that these PAC are well suited to rank animals based on their methane production levels, and to determine relative differences between experimental groups. Importantly, in genetic trials a more precise measurement or phenotype can only improve the precision of the breeding value used to rank animals for selection. Precision of the breeding values can also be increased through increasing the number of relatives which are also measured (Lassen and Difford, 2020), thus increased throughput of the method also directly improves the precision of the breeding value, even though it does not affect the precision of the direct measurement.

Additionally, we detected a significant shift in accuracy which follows a linear trend across the measurement range. This phenomenon is commonly observed with gas analysers using infrared spectroscopy, whose measurement accuracy is known to drift across the concentration range. The resulting absolute measurement accuracy appeared to be insufficient for studies requiring high measurement accuracy. In principle, PAC could potentially be used for applications requiring high measurement accuracy if the proper recovery testing and calibrations are followed, as outlined in this study. However, work is needed to establish the relationship between the short term PAC measurement 50 min its relationship to the full 24 hour period it is approximating in the presence of factors like diurnal and postprandial variations.

Importantly, these results only reflect the performance of the tested units and in the absence of extraneous biological variations due to animals. In practice, researchers conducting trials using PAC need to employ suitable experimental designs to control or remove these factors.

Lastly, ring and range recovery tests are an attractive approach to harmonising different generations of PAC or even other methods of measurement when researchers can benefit from combining databases. For example, joint genetic evaluations and GHG inventories have received considerable attention in dairy cattle (Garnsworthy et al., 2019; Manzanilla-Pech et al., 2021) and is anticipated to receive similar research inputs in small ruminants in the future.

2.5. Conclusions

The portable accumulation chambers tested in this study achieved recovery rates between 67.6 and 74.5%, with a statistically significant negative linear shift in recovery across the methane range. For applications investigating absolute differences in MPR, the current measurement accuracy is insufficient. However, conducting recovery tests across the tested methane mass range can be used to calibrate measurements and resolve shifts in accuracy. The scope of PAC applications could therefore be extended to applications requiring higher accuracy, provided that these calibrations are conducted rigorously and repeatedly. The chambers showed very high precision, with a repeatability coefficient of 0.99 and high linear correlation with the true values of methane mass (>0.99). This confirms that the PAC included in this study are well suited for applications looking at the relative difference between individuals or experimental groups, such as ranking or breed comparisons.

Disclosures

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CH4

Chapter 3.

Modelling enteric methane production of dairy cows: a hierarchical Bayesian stochastic approach

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Highlights

- Using climate respiration chamber data, the hierarchical methane rate model can accurately predict the methane production of individual cows.
- This model allows to model population-level shape parameters while allowing for individual variation in scale.
- The partially pooled approach taken proved to make more accurate methane production predictions than its fully pooled equivalent.

Abstract

Monitoring methane production from individual cows is required for evaluating the success of greenhouse gas reduction strategies. However, converting non-continuous measurements of methane production into daily methane production rates (MPR) remains challenging due to the general non-linearity of the methane production curve. In this paper, we propose a Bayesian hierarchical stochastic kinetic equation approach to this challenge. Modelling was used to fit a non-linear curve on climate respiration chamber (CRC) data of twenty-eight individual dairy cows before computing an area under the curve, thereby providing an estimate of MPR from individual cows. The shape parameters of this model were pooled across cows (population-level), while the scale parameter varied between individuals. This allowed for the characterization of variation in MPR within as well as between cows. Model fit was thoroughly investigated through posterior predictive checking, which showed that the model could reproduce the CRC data of twenty-eight cows well. Comparison with a fully pooled model (all parameters constant across cows) was evaluated through cross-validation, where the Hierarchical Methane Rate (HMR) model proved to perform better. Concordance between the values observed in the CRC and the ones predicted by the HMR model was assessed with R^2 (0.995), r (0.997), root mean square error (10.0 g/d), and Lin's concordance correlation coefficient (0.961). Overall, the predictions made by the HMR model appeared to reflect individual MPR levels and variation between cows as well as the standard approach taken by scientists with CRC data.

3.1. Introduction

Livestock farming faces an urgent need to reduce its greenhouse gases (**GHG**) emissions and reduce the impact of the sector on the environment. Methane (**CH_4**) is a major GHG contributor, and its production by ruminants - and particularly cattle - requires considerable reduction.

Methane is produced by ruminants as a result of the enteric fermentation process that occurs in the digestive tract during the anaerobic fermentation of feed. During this process, carbon dioxide (**CO_2**) and other carbon-containing metabolites are produced and reduced into CH_4 by the methanogens (Morgavi et al., 2010) before being released by the animal through exhaling and eructating.

To mitigate enteric methane production at cow level, several reduction strategies have been identified. These mainly involve dietary modifications or the breeding of individuals with lower methane production levels (Arndt et al., 2022; Hristov et al., 2013b; Knapp et al., 2013). However, evaluating the success of these strategies requires the availability of methods that can accurately quantify the methane production rate (**MPR**) of individual cows under practical conditions (*e.g.* while housed in barns or on pasture). Although several approaches to measure methane production under these conditions have already been developed (*e.g.* GreenFeed, sniffers, use of SF_6 as a tracer gas), they all present challenges with respect to measurement accuracy and throughput, animal intrusiveness, practicability, and costs (Hammond et al., 2016a; Negussie et al., 2017). Additionally, MPR vary in time and in response to feeding. For example, dietary composition, dry matter intake (**DMI**), and frequency of feed intake affect postprandial (subsequent to feed intake) production rates (Hristov et al., 2013b; K. A. Johnson and Johnson, 1995). Ruminal fermentation rate is time dependent, with postprandial durations affecting MPR in a non-linear manner. A rapid rise towards a methane production peak (reached 30 to 140 min after feeding) and a slow decrease back to the basal production level (Crompton et al., 2011; van Lingen et al., 2017) can be observed in cattle, the amplitude and period depending mainly on feed intake pattern. As a result, accurate MPR estimations necessitate the entirety of the methane production curve to be monitored or known in a reliable way.

Practical monitoring techniques only allow for spot sampling as they are often limited by material and labour resources, or rely on the voluntary visit of animals to the monitoring device. They may result in observations that are not well distributed over time, and are prone to bias (Hammond et al., 2016a). Given the postprandial variation in MPR, measurements should cover complete periods between two feed allocations or meals, and not rely solely on scarce spot

sampling to accurately estimate methane production (Hammond et al., 2016a; Kuhla et al., 2015). The number of spot samples required for accurate estimates of daily methane production depends on factors including frequency and level of feeding (Lee et al., 2022; van Lingen et al., 2023). In order to convert discrete (non-continuous) measurements of methane productions into daily MPR, modelling could be used. The output collected by methane measurement devices could be used to fit a non-linear curve before computing the area under the curve, therefore providing an estimate of MPR.

Numerous models have been developed to simulate postprandial methane production from dairy cows. These include static empirical models that directly relate nutrient intake and methane output, and dynamic mechanistic models that use mathematical descriptions of ruminal methanogenesis to predict methane production (Mills et al., 2003). Whilst some of these models offer a very complete computation of physiological processes (Bannink et al., 2011; Kebreab et al., 2019; van Lingen, 2017), they usually require extended diet-related information as input to the model (*e.g.* DMI, metabolizable energy intake, and dietary starch, cellulose, and other carbohydrates content). However, such information is not always available in practice, emphasizing the continued need for and interest in basic modelling that necessitates less information as input.

A more straightforward approach was taken by Crompton et al. (2011), who fitted dairy cattle MPR measured by climate respiration chambers (**CRC**) as an exponential response to feeding frequency and postprandial duration. Their model fitted the CRC data satisfactorily and described well the fluctuations in methane release in response to the changes in feeding pattern. However, the Crompton et al. (2011) model was designed to be fitted to individual cows. While information could also be pooled over cows, it would prevent the borrowing of information across cows, implying that the variability in MPR could be either overestimated (in the case of individual fitting) or underestimated (with pooled fitting) (Gelman and Hill, 2006). Moreover, the usual nonlinear regression approaches to fit kinetic models to observed data are limited in their flexibility and propagation of uncertainty (van Boekel, 2022).

The objective of the current work was to extend the model of Crompton et al. (2011) using a Bayesian hierarchical stochastic kinetic equation approach. The hierarchical model we propose is partially pooled, having population-level shape parameters while also carrying a cow-level scale parameter. This approach allows for flexibility in the modelling of MPR over multiple cows. Moreover, the model emphasizes uncertainty propagation, with the intention of realistically representing variation in MPR between cows. In the remainder we first describe

the basic model and its Bayesian hierarchical extension. We will also explain our approach to the estimation of the model's parameters. Next, we include and discuss results regarding model fitting, assessment, and comparison with a CRC dairy cattle dataset.

3.2. Material and methods

3.2.1. Dataset

The database used to fit the proposed model was obtained in a study conducted from August to October 2020 at the animal research facilities of Wageningen University & Research (Wageningen, the Netherlands) under the Dutch Law on Animal Experiments and in accordance with EU Directive 2010/63. All experimental procedures were approved by the Central Committee of Animal Experiments (The Hague, the Netherlands; 2017.D-0079.004).

The experiment was conducted with 28 lactating Holstein-Friesian cows (mean 2.3 ± standard deviation 0.9 lactations; 93 ± 27 days in milk, **DIM**). Cows were blocked (7 blocks of 4 cows) based on parity and DIM and fed a basal total mixed ration (**TMR**) throughout the entire study consisting of 41% corn silage, 32% grass silage, and 27% concentrate on a dry matter (**DM**) basis. The TMR was formulated to meet 100 and 95% of net energy for lactation and metabolizable protein requirements (CVB, 2018), respectively, for cows consuming 22 kg DM/d and producing 34 kg/d of milk containing 4.0% fat and 3.4% protein. Each block was housed separately in a free-stall barn to facilitate ad libitum feed intake measurement of the 4 cows within each block. The average feed intake of the block during the final 3 d of the 7-d free-stall ad libitum intake period was used to set a fixed daily feed allocation for individual cows within the block. This fixed amount (equal for all cows within a block) was fed individually during a 12-d tie stall period and a 4-d measurement period in CRC.

After 12-d of adaptation to movement restriction in tie stalls and fixed amount of feed allocation, cows were moved into CRC for a 4-d measurement period to facilitate determination of gaseous exchange. The design and principles of the CRC at Wageningen University (Wageningen, the Netherlands) have been described in detail by Van Gastelen et al. (2015) and Heetkamp (2015). The CRC consist of two large chambers, each divided into two sub-compartments (total of 4 CRC, each with a ground surface of 11.8 m^2, and a volume of 34.5 m^3) separated by airtight walls. Walls are equipped with windows, which allow cows to see and hear each other to minimize the impact of isolation on cow stress level, behaviour, and

performance. During the experiment, the relative humidity and temperature were maintained at 80.1% (± 1.2) and 10°C (± 0.1), respectively, by two computer-controlled air conditioning units. In each individual CRC, relative humidity was monitored by one Novasina Hygrodat100 sensor (Novasina AG, Lachen, Switzerland) and temperature was monitored by five PT100 sensors (Sensor Data BV, Rijswijk, the Netherlands) evenly spread over the chamber. Outside air was continuously pumped into the chamber at a ventilation rate of 43 m^3/h (± 1.1) by a gas volume meter (Itron Delta 2080 G100, Itron GmbH, Karlsruhe, Germany). Exhaust air was released through a conduit equipped with an iris valve, allowing the constant regulation of the ambient pressure at a value of 102.9 kPa (± 7.36). Inlet and outlet air of each compartment was sampled for its CH_4, CO_2 and O_2 content to be analysed by a series of ABB Advance Optima AO2000 analysers (ABB, Berlin, Germany). The analysis of the CH_4 and CO_2 concentrations was done using a nondispersive infrared method, and a paramagnetic one for O_2 concentration. Before the start and after the end of the experiment, the recovery rate of each CRC was assessed by releasing known amounts of CO_2 and CH_4 and comparing them to the ones measured by the gas analysis system. The average recovery of CO_2 was 100.1% ± 0.4 (ranged from 99.4 to 100.5%), and the average recovery of CH_4 was 100.8% ± 0.5 (from 99.1 to 102.1%). During the experiment, the four CRC shared the same gas analysers in sequence, and the CH_4 and CO_2 production and O_2 consumption of each chamber was computed every 12 to 15 min by calculating the difference between inlet and outlet gas volumes and concentrations, and corrected to standard temperature and pressure. Automatic valves redirected the sampled air of a compartment to the analysers, flushing it for 120 s and recording the concentrations for the last 30s before moving to the next chamber or to inlet air.

Milking and feeding occurred twice daily during the entire experiment in the CRC (0500 and 1530 h). Gas measurements during time points when staff entered the CRC compartments for milking and feeding (maximum 30 min) were maintained in the dataset as humans do not emit significant quantities of CH_4 and because including CH_4 measurements during these time points, compared with excluding these CH_4 measurements, did not affect the daily production of CH_4 (van Gastelen et al., 2017). Postprandial durations during the measurement period were calculated as the time differences from the feeding times to the occurrence of each gas sample, giving values between 0 and 13.5-h. For the purpose of the study, MPR that were not monitored over a complete 24-h period were removed from the dataset as they provided biased estimates. Thus, data from 1530 on d 1 in the CRC until 1530 on d 4 in the CRC was used.

For the purpose of the separate study not related to the current analysis, cows were randomly assigned to treatments which consisted of the basal TMR or the basal TMR including 1 of 3 iso-MP rumen-protected protein supplements. The supplements were mixed by hand into the TMR at each moment of fresh feed allocation for the final 16-d of the experiment (12-d tie stall period and 4-d measurement period in CRC). These treatments did not affect DMI (mean = 18.7 kg/d; SEM = 0.63; p = 0.91) or methane production over the 4-d period in the CRC (mean = 185 kJ/kg $BW^{0.75}$/d; SEM = 5.5; p = 0.35).

3.2.2. Crompton et al. (2011) kinetic model

In the study of Crompton et al. (2011), the MPR of 4 lactating Holstein-Friesian cows were monitored in open-circuit CRC over 4-d. Cows were fed either 1, 2 or 4 times daily. Crompton et al. (2011), following the principles introduced in the lactation curve described by Dijkstra et al. (1997), defined a mathematical model which reuses the Gompertz growth function (Gompertz, 1825) with the addition of a decay term. This resulted in the following exponential function to describe asymmetrical shape MPR profiles:

$$M\ (g/d) = \alpha \cdot \exp\left[\beta \cdot \left(\frac{1 - e^{-\gamma t}}{\gamma}\right) - \delta t\right] \qquad \text{[eq. 3. 1]}$$

where M is the postprandial MPR, α is a scale parameter representing the theoretical MPR at t = 0, β is a proliferation parameter, and γ and δ are decay parameters. Hence, β, γ, and δ are shape parameters. All parameters must be non-negative.

This model is based on the assumption that after ingestion of a meal and up to ingestion of the next meal, methane production is deterministically characterised by a phase of rapid increase up to a maximum value, and a following decreasing slope back to the basal level (Crompton et al., 2011; Macciotta et al., 2011). However, individual deviations from the standard shape exist (*e.g.* due to intake pattern, diet composition, and genetics). These deviations represent a stochastic component that induces individual deviations from the standard population curve, and which we desired to implement in the model. Additionally, individual MPR computations offer limited scaling possibilities as the obtained parameters values are only valid for the individual cow for which they were computed and cannot be extrapolated to other cows.

3.2.3. Hierarchical Methane Rate model

To generalize the model of Crompton et al. (2011) and extend its applications, we reformulated it into a partially pooled multilevel (hierarchical) model. This so-called Hierarchical Methane Rate (**HMR**) model is a Bayesian representation (Appendix C) of a stochastic process that aims at simultaneously integrating the data of multiple individuals to model the grand MPR curve of that specific population in the specific housing and management conditions. From this estimated population curve, 3 shape parameters (β, γ, and δ) can be derived and generalized to the studied population, whilst the scale parameter (α) is allowed to vary over cows. Individual MPR curves can then be derived from the estimated population curve by allowing each cow to diverge from the original curve, through the inclusion of variation in α and measurement errors.

Being partially pooled, the HMR model is a compromise between the principles of pooled and un-pooled models. It allows for the information from different repetitions (cows) to be connected and combined (like pooled models), while still allowing for each individual to vary from the rest of the group (multilevel modelling). Group means are thus considered as being a random sample of a population, and the parameters of the model can be sampled from a single distribution (van Boekel and Roux, 2022). Multilevel modelling also has the advantage of counteracting the risks of pseudo-replication, cases that lead to an underestimation of the variation and to biased estimates (Lazic et al., 2020, 2018).

The HMR model likelihood is therefore defined as methane per cow (M_c):

$$M_c \sim N(\mu_c, \sigma_\epsilon)$$

With

$$\mu_c\ (g/d) = (\overline{\alpha} + \alpha_c)\ \cdot \exp\left[\beta \cdot \left(\frac{1 - e^{-\gamma t}}{\gamma}\right) - \delta t\right] \qquad \text{[eq. 3. 2]}$$

In this model, we have a population scale effect $\overline{\alpha}$ as well as a random scale effect for cow α_c. This is thus a *partially pooled model*, where the shape parameters are population parameters, and the scale parameter carries a random effect for cow.

We formulated the following prior distributions:

$$\bar{\alpha} \sim N(500, 200)$$

$$\alpha_c \sim N(0, \sigma_\alpha)$$

$$\beta \sim N_+(0, 2)$$

$$\gamma \sim N_+(0, 2)$$

$$\delta \sim N_+(0, 2)$$

$$\sigma_\alpha \sim T_3^+(0, s)$$

$$\sigma_\varepsilon \sim T_3^+(0, s)$$

where N_+ denotes the half-normal distribution, and T_3^+ indicates a half t-distribution with 3 degrees of freedom. The σ_α parameter represents the between-cows variance in the scale parameter, while σ_ε characterizes the within-cow variance in the response variable. The scale factor s is set to the pooled median deviation of observed methane over time. This is a robust choice. These priors reflect the nonnegativity requirement on the shape and variance parameters. Moreover, they are weakly informative while supportive of sampling efficiency (Bürkner, 2017).

3.2.4. Computational approach and convergence assessment

We generated samples from the posterior distributions of the parameters in the HMR model by way of Hamiltonian Monte Carlo (Bürkner, 2017). For each parameter we ran 4 Markov chains of 2000 iterations. The first 1000 iterations were discarded as warm-up, leaving 4000 iterations per parameter for inference. In the Bayesian approach, estimation then concurs with summary statistics of the posterior draws while inference concurs with the evaluation of proportions of posterior draws. We applied various convergence diagnostics for our sampling approach. First, we visually inspected the multiple, randomly started Markov chains for convergence on the same stationary distribution. In addition, we calculated the Gelman-Rubin convergence diagnostic (denoted $\hat{R}$) (Gelman and Rubin, 1992). It compares, for each parameter, the estimated between-chains and within chain variances. Intuitively, a well-mixing, stationary set of chains will have within-chain variances approximately equal to the between-chains variance. As a rule-of-thumb, an $\hat{R} \leq 1.01$ (Vehtari et al., 2020) or 1.1 (Gelman and Rubin, 1992) is taken as evidence for convergence.

3.2.5. Model diagnostics and model comparison

The goodness of fit of the HMR was assessed using Posterior Predictive Checks (**PPC**) and their related Bayesian p-values. The purpose of PPCs is to determine whether a model adequately describes the collected data or not (Gelman et al., 1996; Lynch, 2005). It allows to evaluate the predictive performance of a model by replicating data under this fitted model (which is a representation of data we might collect in the future) and comparing its outcome to the actual observed values. If the model adequately represents the data-generating mechanism, then replicated data under the posterior model will behave as the observed data. In the current study, a PPC was performed by generating 1000 draws of data from the posterior distribution of the fitted model and comparing its outcomes to the observed values to look for systematic discrepancies. In complement, a Posterior Predictive Distribution (**PPD**) analysis was performed. The PPD shows the distributions of future plausible observations under the fitted model and, therefore, whether or not the mean and 99th quantile of the replicated data are within the range of the observed values.

The HMR was compared to a fully pooled model with respect to their predictive performances. This comparison uses a leave-one-out cross-validated (**LOO-CV**) estimate (Vehtari et al., 2017) of the expected log predictive density (**ELPD**). The ELPD is the expected log pointwise predictive density for a new observation, *i.e.* it is indicative of the expected predictive accuracy for new data. Higher ELPD scores indicate better models. Models can be compared based on their ELPD difference. An absolute difference in ELPD higher than 4 indicates that one model has notably better predictive performance than the other model (Sivula et al., 2020). The associated standard error (**SE**) reflects the level of uncertainty with respect to the ELPD difference: if the SE is small relative to the difference, we are more certain that the models under comparison do differ in their predictive performance levels.

The comparison of the HMR model with a fully independent equivalent (no pooling) was not investigated in this LOO-CV. A no pooling approach would have led to as many models as there are repetitions (van Boekel and Roux, 2022), *i.e.* 28 models. Therefore, each LOO-CV computation would only provide insight on the balance of one of the 28 models against HMR, and general conclusions on balance and goodness of fit could not be drawn. Most importantly, fully independent modelling yields estimates of the parameters that are not connected to the parameters' values obtained for the other individuals of the group (van Boekel and Roux, 2022). As a result, group means are approached independently, as if there was an infinitely large variation between groups. This tends to overestimate the differences between groups, leading

to overfitting (Gelman and Hill, 2006). Comparison with such a modelling approach was therefore not done.

3.2.6. Concordance metrics

After derivation of the individual MPR curves, their areas under the curve (**AUC**) were computed to provide the methane production of a cow over time. The AUC were calculated as follows:

$$\mathrm{AUC_c} = \int_0^{t_f} \widehat{\mathrm{M_c}}\, \mathrm{dt} \qquad \text{[eq. 3. 3]}$$

where $\widehat{M_c}$ represents the predicted (based on our Bayesian posterior estimates) methane production curve for cow *c* and where t_f represents the final measurement time. This integral is non-analytical and we used standard numerical integration techniques for its evaluation.

The AUC of each cow was then converted into a daily MPR (g/d) by dividing its value by the time fraction over which it was computed (duration between two feed allocations).

For each cow, methane production assessments conducted over the relevant 3-d in the CRC were averaged into a single daily MPR estimate, as is common practice in CRC research. These individual values represent the reference value against which the model predictions were compared. Measured (averaged) and predicted MPR curves were plotted for each cow.

A linear regression of the CRC' measurements on HMR' predictions was performed to test for the presence of a linear relationship between averaged CRC and predicted HMR estimates. It was used to determine R^2, which indicates the proportion of variance in the response variable that is explained by the regression model. Pearson's correlation coefficient (r) was derived from R^2, which denotes the level of correlation between the observed and predicted MPR. Both R^2 and r yield values between 0 and |1|. In addition, the Root Mean Square Errors (**RMSE**) were calculated to measure the prediction errors made by the model, with smaller values reflecting a lower level of error.

The core of the HMR model is to sense variations in MPR between individuals. Therefore, agreement between observed and predicted MPR estimates is sought. To assess this level of agreement, we used Lin's concordance correlation coefficient (**CCC**) (Lin, 1989), taking a value between -1 (perfect disagreement), 0 (no agreement) and 1 (perfect agreement).

3.2.7. Software and code

The HMR model was coded in R (x64 4.1.0.) using the public libraries *Bolstad2* (Bolstad, 2010), *brms* (Bürkner, 2017), and *Rcpp* (Eddelbuettel and Francois, 2011). The leave-one-out-cross-validation was done using the *loo* library (Vehtari et al., 2017).

3.3. Results and discussion

3.3.1. Computational approach and convergence assessment

The posterior means of the α, β, γ, and δ parameters were extracted from the inference analysis. Across the range of cows, $\overline{\alpha}$ = 242.1 ± 7.7, β = 2.38 ± 0.15, γ = 2.99 ± 0.11, and δ = 0.0393 ± 0.0005 (Table 3.1). The variability between cows for the individual scale parameter $\overline{\alpha}$ equated to $\sigma_{\overline{\alpha}}$ = 21.88 ± 3.37, which reflects the variability that is naturally present between cows and the ability of the model to reflect it. Deviation in the scale parameter values between cows is shown in Figure 3.1.

For each of the estimates, the convergence diagnostic $\hat{R}$ indicates the convergence of the Hamiltonian Monte Carlo. As all values are lower than or close to the rule of thumb 1.01 (Vehtari et al., 2020) or 1.1 (Gelman and Rubin, 1992), we can conclude that the chains converge. This means that a stationary distribution (equilibrium) has been reached and that no convergence issues were detected, indicating that the estimates of the parameters can be trusted (Gelman and Rubin, 1992).

Table 3.1. Bayesian estimates of the population parameters $\bar{\alpha}$, β, γ, and δ and variance components $\sigma_{\bar{\alpha}}$ and σ_{ε}. Estimates are presented with their standard error (SE) as well as lower and upper 95% credible intervals. For each parameter the estimated convergence diagnostic $\hat{R}$ reflects the convergence of the Hamiltonian Monte Carlo chains.

Parameter	Mean	SE	95% Credible interval		$\hat{R}$
			Lower	Upper	
$\bar{\alpha}$	242.1	7.7	227.7	257.7	1.02
β	2.38	0.15	2.10	2.69	1.01
γ	2.99	0.11	2.78	3.21	1.01
δ	0.0393	0.0005	0.03923	0.03926	1.00
$\sigma_{\bar{\alpha}}$	21.88	3.37	16.32	29.71	1.00
σ_{ε}	64.99	0.47	64.07	65.93	1.00

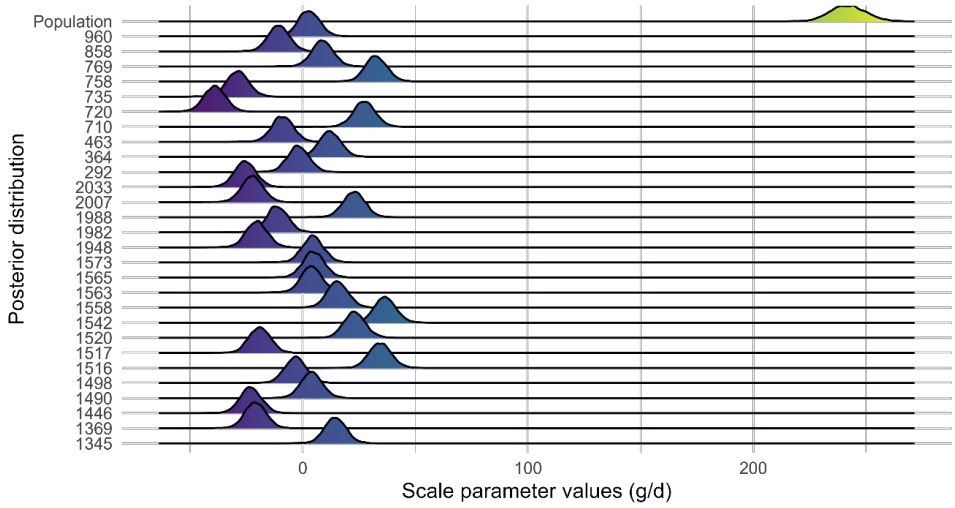

Figure 3.1. Ridgeline plot of the population scale factor ($\bar{\alpha}$) and the individual deviations from the population per cow (α), indicated by their number.

3.3.2. Model diagnostics and model comparison

A 1000 draws of data were replicated in a PPC under the HMR model (Figure 3.2. A). The similarities that can be observed between observed data and samples generated from the PPD (centred, symmetrical) show the predictive capacity of the HMR model and indicates no systematic discrepancies. In addition, the bulk of the distributions of the mean values fell in the middle of the PPD (Figure 3.2. B), which shows the ability of the model to reproduce mean values accurately. The PPD of the 99th quantile of values appeared to lie in the centre of the distribution (Figure 3.2. C), which means that the extreme values are also well represented by the model. Complementarily, the computation of the Bayesian p-values showed that 51.5% of the replicated values of the mean, and 33.6% of the replicated values of the 99th quantile lie within the observed values. According to the rule of thumb, all Bayesian p-values above 0.1 (10%) are acceptable (Lambert, 2018).

In contrast to other metrics, the PPC and PPD methods do provide indications about the predictive capacity of a model. Therefore, these results are particularly informative considering that the purpose of the HMR model is to aid in predicting MPR from new sets of cows.

The LOO-CV was used to assess and compare the predictive performance of the partially pooled model (HMR) with that of its fully pooled equivalent. The fully pooled model yielded an estimated ELPD of -53411.4 (SE = 90.2), while the ELPD of the partially pooled model

equalled -51758.5 (SE = 85.9). As the difference in ELPD of 1652.9 is significantly larger than |4| and in favour of the partially pooled model, it shows that the HMR model predicts the observed data significantly better. The associated SE difference of 69.3 corresponds to 4.2% of the difference in ELPD, which reflects the low uncertainty that the difference in prediction accuracy between the two models could have been due to chance. These results confirm that the partially pooled approach taken with the HMR model makes it more suitable to predict individual MPR from cows.

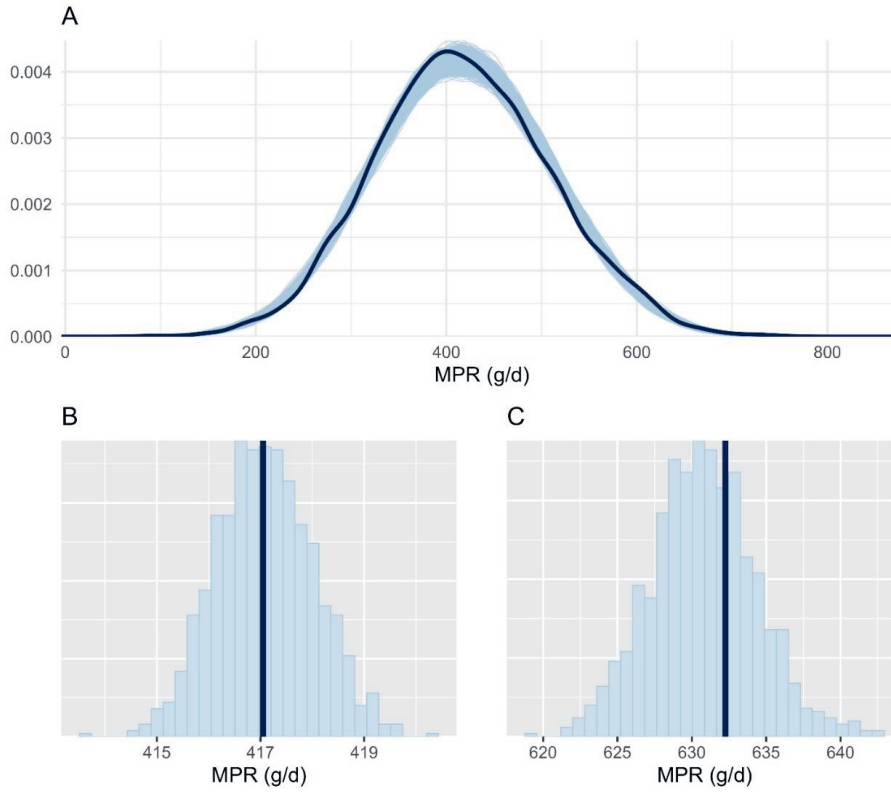

Figure 3.2. Posterior Predictive Checks (PPC) of the HMR model. A: PPC of the replicated data (1000 draws) fitted with the HMR model for the studied grand population of cows. Dark plain line: observed values; light plain line: replicated values. B: Posterior Predictive Distribution (PPD) of the mean of the values fitted with the HMR model (1000 draws) for the studied grand population. Dark plain line: observed mean; bars: replicated means. C: PPD of the 99th quantile of the values fitted with the HMR model (1000 draws) for the studied grand population. Dark plain line: observed value of the 99th quantile; bars: replicated 99th quantile values.

3.3.3. Concordance metrics

The prediction of the grand population's MPR by HMR resulted in one estimated methane production trendline (Figure 3.3. A), complemented by the upper and lower bounds of its credibility interval (0.95). Individual MPR curvatures were derived from the population prediction, two examples of which can also be seen in Figure 3.3 (B and C). An AUC was estimated for each cow and converted into MPR, resulting in a mean MPR of 407.2 ± 35.0 g/d (coefficient of variation CV = 8.6%, Table 3.2).

The MPR measurements done by the CRC over 3-d were plotted for each cow. Figure 3.4 shows the curves of two example cows, with the other individuals providing similar standard curves, varying only in range. All methane production measurements collected over the 3-d in the CRC were averaged into a single daily MPR estimate per cow, as is common practice in CRC research. In the CRC, mean daily MPR was 416.7 ± 36.2 g/d (Table 3.2), and variability between cows 8.7%.

Despite the fact that the model must deal with the uncertainty resulting from the borrowing of information from a population that is propagated to individuals, the mean MPR and inter-cow variability predicted by the HMR model appeared in agreement with the levels monitored by the CRC. Additionally, the goodness of fit of the HMR model was first assessed through the linear regression of the MPR that were monitored and predicted for each of the cows (Figure 3.5). It resulted in a R^2 value of 0.995 (Table 3.3), which shows the strong ability of the model to explain the variance in monitored MPR. A value of 0.997 was obtained for Pearson's r, which reflects the strong and nearly perfectly linear correlation between the observed and predicted MPR (Altman, 1990). The RMSE equalled 10.0 g/d, which is 2.41% of the observed (monitored) mean. This value reflects a high prediction accuracy. Lastly, the computation of Lin's CCC resulted in an excellent concordance (0.961). This result shows the ability of HMR to predict the MPR of individual cows, which is essential for its future applications.

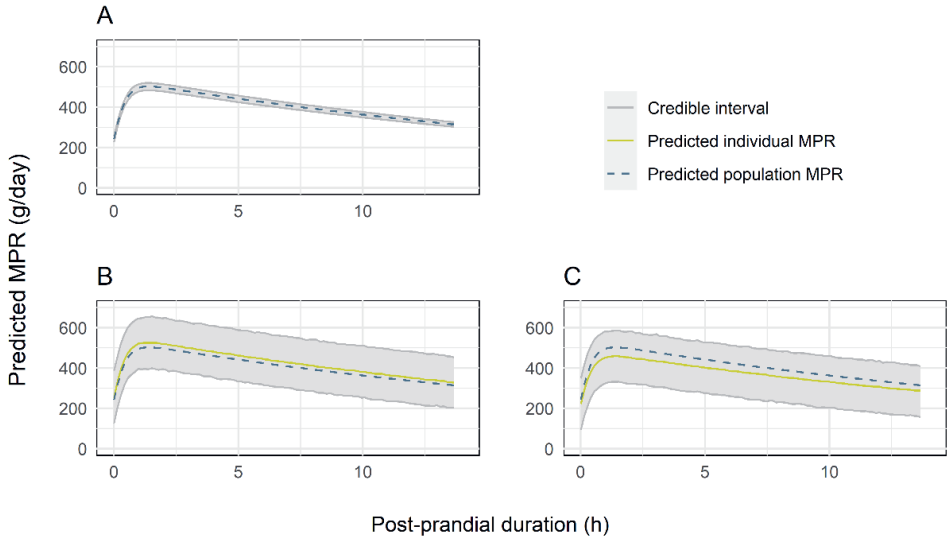

Figure 3.3. Postprandial MPR as predicted by the HMR model. A: Prediction of the grand population curvature. B, C: Example of two individual curves (cows 2 and 12) as deduced by the HMR model from the predicted grand population curvature. Grey ribbons: credible intervals; dashed lines: predicted MPR of the grand population; plain lines: predicted MPR of individual cows.

Table 3.2. Mean methane production rate (MPR), standard deviation (sd) and coefficient of variation (CV) between cows as monitored by the respiration chamber and predicted by the Hierarchical Methane Rate (HMR) model.

Monitored			Predicted		
Mean individual MPR (g CH_4/day)	sd (g CH_4/day)	CV (%)	Mean individual MPR (g CH_4/day)	sd (g CH_4/day)	CV (%)
416.7	± 36.2	8.7	407.2	± 35.0	8.6

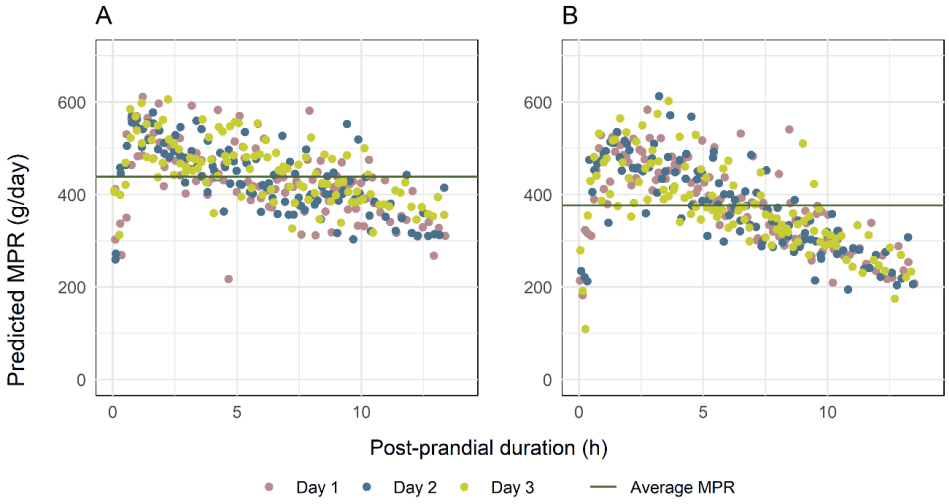

Figure 3.4. Example of two individual MPR (g/d) monitored by the CRC over a total period of 3-d for cow 2 (A) and 12 (B). Each colour represents the combination of the 2 postprandial MPR monitored over this 24-h time interval (Day 1, Day 2, Day 3). The plain horizontal line represents the mean methane production rate obtained for the monitored cow (g/d).

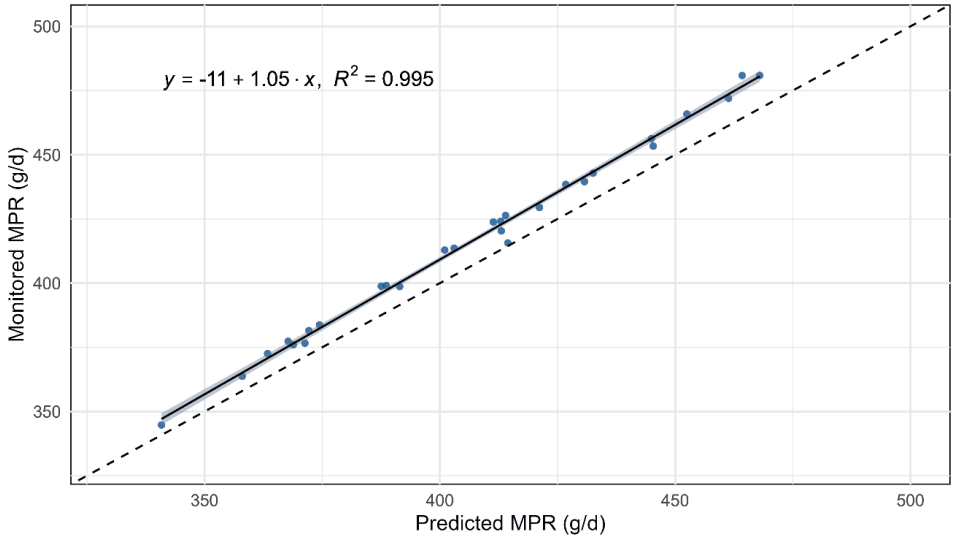

Figure 3.5. Linear regression of the individually monitored (CRC) and predicted (HMR) MPR (g/d) and regression line. Dashed line shows x = y.

Table 3.3. Indicators of the goodness of fit of the model R2, Pearson's r, and root mean square error (RMSE). Estimate, p-value and confidence intervals of Lin's concordance correlation coefficient (CCC).

Indicator						
R^2	RMSE	RMSE as % of the observed MPR mean	Pearson's r	CCC	Lower CI	Upper CI
0.995	10.033	2.407	0.997	0.961	0.933	0.978

3.3.4. General discussion

In this paper, we proposed a Bayesian hierarchical stochastic kinetic model to predict MPR. The hierarchical model we exposed is partially pooled, and has population-level shape parameters while carrying a cow-level scale parameter. This approach allowed for flexibility in the modelling of MPR over multiple cows, therefore allowing the computed curves to reflect part of the variations in MPR that are present between individuals. We emphasized uncertainty propagation, with the intention to realistically represent variation in MPR between cows. We firstly described the basic model and its Bayesian hierarchical extension, before explicating our approach to the estimation of the model's parameters. We assessed and discussed model fit and concordance.

The extensive model evaluation that has been carried out in this paper has shown that the HMR model is an adequate representation of the postprandial MPR curve. Its ability to partially pool information from a grand population of cows and to allow for individual variability in the chosen scale parameter has proven effective. Its predictive performance was shown and gauged to the one of its fully pooled equivalent model. The latter resulted in lower prediction accuracy and underfitting. Placed in a Bayesian context, the HMR model has broadened the applications of the model of Crompton et al (2011).

In a next phase, time could be implemented as an additional hierarchy level of the model. Indeed, cows are subject to changes in physiological status and feed intake over time, which have an impact on MPR. Therefore, implementing this source of variability in the HMR' predictions could improve the model further. Additionally, assessing its goodness of fit and predictive performance under conditions of differing feeding frequencies (*e.g.* once, twice, thrice daily, ad libitum, restricted), lactation status, and milk yield would be very informative

as these factors have the potential to impact methane production levels (Bittante et al., 2018). Besides, feeding frequency is a major factor in the required number of daily samplings and their intervals during CH_4 spot sampling techniques to obtain reliable methane production values of individual animals (Lee et al., 2022; van Lingen et al., 2023). The present HMR model assumes an asymmetrical shape of methane production rate upon consumption of a meal, with a continuous rise to a peak methane production followed by a gradual decline. The number of actual meals of an animal is then a major determinant of the parameters of the HMR model, with for example a more rapid decline after peak production in animals consuming several small meals compared with animals consuming one large meal daily, as demonstrated by Crompton et al. (2011).

Complementarily, the presence of individual variation in the shape parameters could be explored. In fact, the demarcation of the model only allows the scale parameter to vary between cows, whereas shape might also vary between individuals. It would therefore be interesting to study the individual variation of the three shape parameters and assess which of them is most likely to vary from one individual to another. However, such a study will be computationally expensive.

Lastly, it would be interesting to investigate the gain in MPR estimation accuracy obtained when the HMR model is used to fit datasets resulting from monitoring devices that operate at lower sampling frequencies than the CRC, such as the commonly used spot sampling methods used in practice (*e.g.* GreenFeed, sniffers, Cubicle Hood Sampler). Indeed, their throughput is usually limited and does not cover full periods between two feedings. As previously stated, monitoring or modelling the entirety of the MPR curve is key to accurate MPR estimates, which is where the HMR could be of aid for two reasons. First, because it allows to convert discrete measurements into a non-linear production curve that respects the mechanics of postprandial methane production. Secondly, because the borrowing of information across cows could partially compensate for the limited amount of information that is available for one individual. Mathematically, the HMR model is able to operate with limited input information, but its prediction accuracy is expected to vary depending on the number of observations and the phase of the methane production curve at which they have been collected.

3.4. Conclusions

This study demonstrated the ability of the HMR model to predict MPR of individual cows (using CRC data) by modelling population-level shape parameters while allowing for individual variation in scale. The hierarchical Bayesian approach taken with the model proved to make more accurate MPR predictions than its fully pooled equivalent, and to offer broader application opportunities than the previous models. Next, we suggest to fit the HMR model with datasets from spot-sample measurement devices and to investigate its effect on the MPR estimation accuracy.

Disclosures

The authors declare no conflicts of interest financial or otherwise.

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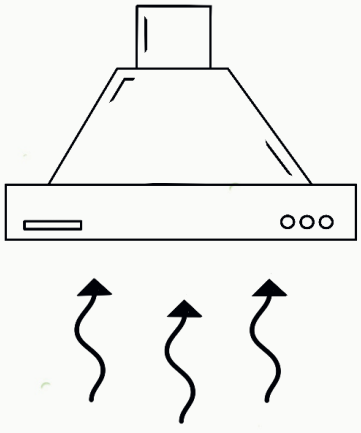

Chapter 4.

Optimisation of the Cubicle Hood Sampler for practical monitoring of enteric methane

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Highlights

- Ultrasonic sensors that are not dust-tight and waterproof are not suited for use in barns.
- As used here, the performance of radio frequency identification was not sufficient.
- Ultrasonic sensors and radio frequency identification will be replaced by computer vision.
- The recovery tests conducted showed the system's ability to monitor methane production accurately.

Abstract

Monitoring methane production from individual cows is crucial for the implementation of greenhouse gas reduction strategies. However, monitoring methane production rates (**MPR**) under practical conditions and with acceptable levels of accuracy, intrusiveness, and throughput remains challenging. In this study, we present a renewed design of the Cubicle Hood Sampler (**CHS**) as an alternative solution to this challenge.

Placed in the cubicles, the CHS collects and analyses the methane content of the air exhaled by cows when lying down. Ultrasonic sensors were used to monitor the head position of cows within the system, information of importance when measuring breath components. However, they appeared not to be suited for barn use. Radio frequency identification was used to link measurements to specific cows but, as used here, the levels of correct identification were insufficient. The ability of four CHS to recover known MPR was assessed in three series of recovery tests using a reference method (artificial reference cow (**ARC)**). For the tested fluxes, there were no significant difference in recovery rates (mean 110.5% ±8.7) between CHS (p = 0.207), treatments (p = 0.080), and repetitions (p = 0.148). The coefficients r = 0.99, and R^2 = 0.98 showed that the correlation between injected and recovered fluxes was strong and positive, and that variance in recovered rates could be well explained by variance in the injected values. Repeatability equated 0.94, showing the excellent repeatability and reliability across replicates. These results overall place the CHS as a promising tool for on-barn methane measurements.

4.1. Introduction

Currently, livestock farming faces an urgent need to reduce its greenhouse gases (**GHG**) emissions and reduce the impact of the sector on the environment. Methane (**CH_4**) accounts for one of the major GHG contributors, and its production by ruminants (and particularly cattle) requires a considerable shrinkage.

Methane is produced, by all ruminants, as a result of the enteric fermentation process occurring in the digestive tract and during the anaerobic fermentation of feed. During this process, carbon dioxide (**CO_2**) is produced and reduced into CH_4 by the methanogens (Morgavi et al., 2010) before being released by the animal through exhaling and eructating. This by-product is not only a substantial source of GHG, but also an energy loss for the animal (reduction of the feed conversion efficiency) and an economic waste for the farmer (González-Recio et al., 2020; K. A. Johnson and Johnson, 1995).

Mitigation strategies have already been identified to mitigate methane production at cow level. They have as main axis of action the modifications of the rations content, like implementation of additives, reduction of fibre content, increase of feed efficiency (Boadi et al., 2004; Knapp et al., 2013) or the long-term breeding of individuals with lower individual methane production (Clark, 2013; Moss et al., 2000). However, evaluating their performance requires the availability of devices that can accurately quantify the methane production rates (**MPR**) of individual cows throughout time. Although several approaches have already been developed (*e.g.* GreenFeed, sniffers, use of SF_6 as a tracer gas), none of them yet offers an acceptable combination of measurement accuracy, intrusiveness, practicability, and price, while also operating at sufficient throughput (Hammond et al., 2016a; Negussie et al., 2017). In addition, MPR has already been demonstrated to vary in time and in response to feeding. For example, dietary composition, dry matter intake, and frequency of feed intake affect postprandial (subsequent to feed intake) production rates (Hristov et al., 2013a; K. A. Johnson and Johnson, 1995). The rumination status (fermentation rate) has been shown to be inherent to time, with postprandial durations affecting MPR non-linearly. The enteric production of methane is consistently characterized by a first rapid rise towards a production peak (reached 30 to 140 min after feed intake) and a slow decrease back to the basal production level (Crompton et al., 2011; van Lingen et al., 2017). Therefore, accurate estimates of MPR depend as much on the number of observations that can be collected by the system, as on adequate sampling throughout

the production curve. Simple extrapolations from short monitoring periods to daily MPR estimates can therefore not be accurate (Kuhla et al., 2015).

Introduced by Wu et al. (2016), the Cubicle Hood Sampler (CHS) is an innovative device that (non-intrusively) monitors the individual methane production of cows while present in cubicles. By opting for extended monitoring durations (up to 12 hours per cow per day), the CHS could overcome the effect short sampling times have on the MPR estimations of other alternative methods (Kuhla et al., 2015). In addition, the non-intrusive nature of this tool could allow for simple implementation on barns and high throughput. The additional sensors added to the system (cow identification, head position monitoring) provide innovative ways to collect parallel data, which are used in the MPR calculation. These elements place the CHS system as a promising tool in terms of both accuracy and innovation.

The objectives of this study were to evaluate the ability of the latest updated CHS prototype to measure known MPR, and get a first impression of the performance, applicability and resistance of each sensing system under barn conditions. To achieve this, we built four units of an improved version of the CHS and monitored endurance in time, as a result of barn and weather conditions and animal-induced damage. The performance of the cow identification and head monitoring systems were evaluated through comparisons with ground truth observations. The ability of the system to monitor two levels of methane fluxes was investigated through repeated recovery tests. They provided an indication on the measurement accuracy of each CHS at these two levels.

4.2. Material and methods

4.2.1. Dataset

The dataset was collected during a study conducted from August to October 2020 at the animal research facilities of Wageningen University & Research (Wageningen, the Netherlands) under the Dutch Law on Animal Experiments and in accordance with EU Directive 2010/63. All experimental procedures were approved as complying with the regulations on animal experiments in vigour. They were classified by the Animal Welfare Officer as a non-animal experiment, as referred to in the Dutch Act on Animal Experiments, since the experimental procedures caused less pain or distress than the insertion of a needle under good veterinary practice.

The experiment was conducted with 28 lactating Holstein-Friesian cows (2.3 ± 0.9 lactations; 93 ± 27 days in milk). Cows were blocked (7 blocks of 4 cows) based on parity and days in milk, and fed a basal total mixed ration throughout the entire study consisting of 41% corn silage, 32% grass silage, and 27% concentrate on a dry matter basis. The total mixed ration was formulated to meet 100 and 95% of the net energy for lactation and metabolizable protein requirements (CVB, 2018), respectively, for cows consuming 22 kg dry matter/d and producing 34 kg/d of milk containing 4.0% fat and 3.4% protein. Each block was successively housed in the free-stall barn for a period of 7-d each.

4.2.2. Structure and key components

Based on earlier prototypes (Wu, 2016), four updated CHS units were built at the experimental facility of Wageningen University & Research in August 2020 (Nergena, Bennekom, The Netherlands). The units were placed in adjacent cubicles of the free-stall barn, bounded at one end by the building's structure, at the other by the slatted floor.

The physical separation between the lateral sides of each cubicle (left L, right R, Figure 4.1) was made using vertical Plexiglass panels measuring 115 x 140 cm (height x length). An inner space of 115 x 120 cm was delimited around the end of the cubicles receiving the head and forelimbs of the animals with an additional top (T) and two front (F) panels, inserted between the L and R ones. These physical separations were essential to prevent mixing between gas mixtures originating from the monitored animal and the rest of the herd (*e.g.* breaths and belches of cows in adjacent stalls or present on the slatted floor).

Samples were collected through the F panels, which was constituted of two parallel panes, spaced 10 cm apart for gas circulation. Three horizontal slots of 2 x 120 cm were cut in the Plexiglas layer F that was closest to the cows' snouts, at heights of respectively 25, 55 and 125 cm from panel G; an horizontal panel placed 15 cm above ground level to create a protected space for the placement of sensors. These three inlets allowed exhaled and eructated breath to enter the system.

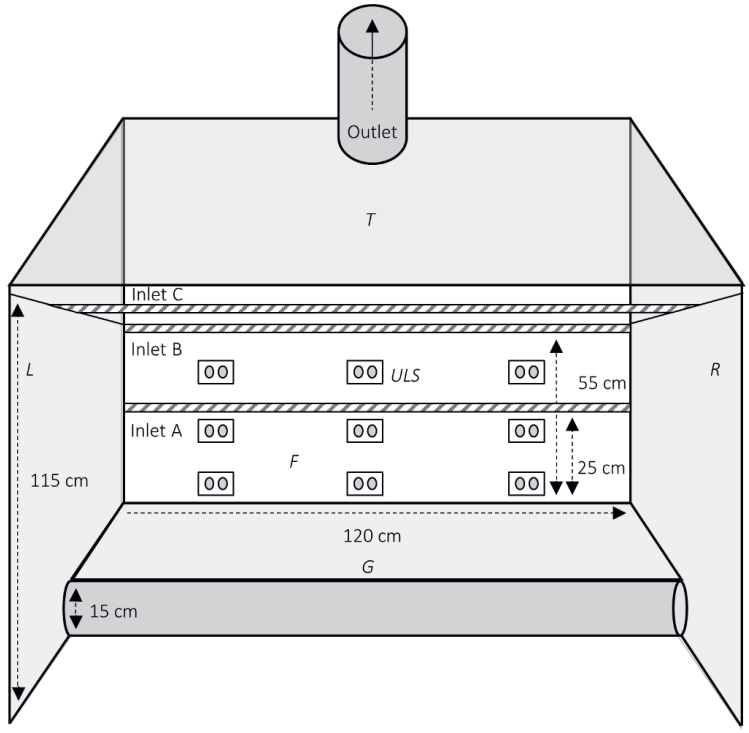

Figure 4.1. Schematic front view of the CHS. Exhaled air flowing into inlets A, B and C (stripped rectangles), and in the direction of the outlet (complete piping not visible). "L" left, "R" right, "F" front, "T" top and "G" ground panels of the structure. "ULS" ultrasonic sensors. Single arrows show airflows, and double arrows represent length.

Placed above the CHS's structure (Figure 4.2), a PVC pipe (⌀ = 110 mm) containing a fan (*4*, TD-250 S&P Holland) permanently induced an airflow of approximately 200 m^3/h. This suction caused an under-pressure in the hood, resulting in the extraction of exhaled air and belches into the piping. Dispersion of the breath air into the barn was limited by the implementation of a rear flap (*6*), falling behind the withers of the cow. In the exhaust, the presence of a flowmeter (*1*, Lambrecht Vane Anemometer 1468) permitted the continuous monitoring of the instigated airflow rate. Temperature and humidity of the sample were monitored using a T-RV sensor (*2*, Vaisala T-RV probe HMP60). Breath samples were collected through a polyethylene tube (*3*, ⌀ = 4.35 mm), filtered for dust particles, and pumped to a gas analyser (Fourier-transform infrated spectroscopy (**FTIR**), Gasmet Technologies) measuring methane concentration. Every 21 seconds, the FTIR provided a mean value of the methane concentration (ppm) measured over the last twenty seconds. All data but the FTIR was collected and stored by a datalogger (CR1000X, Campbell Scientific) every 30 seconds.

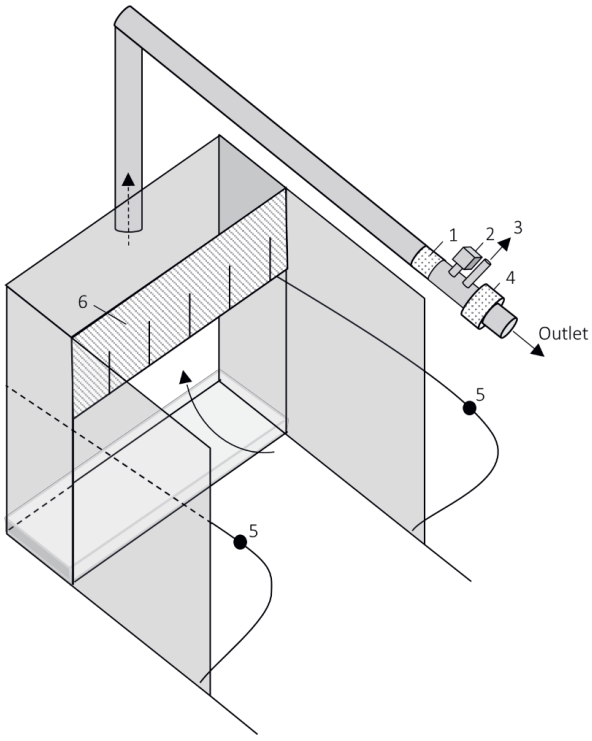

Figure 4.2. Schematic upper side view of the CHS. 1: Flowmeter, 2: T-RV sensor, 3: Hood sampling point, 4: Fan, 5: Background sampling points, 6: Rear flap. Single arrows symbolize airflows.

4.2.3. Sampling strategy and cow recognition

Cubicles equipped with a CHS were monitored in sequence by one FTIR gas analyser. Switching between sampling lines was automated in the datalogger hardware and performed by a multiplexor (engineered and built by Wageningen University & Research Air Quality Lab). The starting and ending of a measurement cycle (**MC**) was based on the recognition of the presence of a cow in one of the units (an example of which is shown in Figure 4.3). Detection and identification of cows (to attribute observations to the correct individual) were carried out using radio frequency identification (**RFID**), consisting of sets of detectable tags (UHF tags, SparkFun Electronics), readable by UHF RFID reader antennas (RP-TNC, SparkFun Electronics). Its coupling to a simultaneous RFID tag readers (Simultaneous RFID Reader - M6E Nano, SparkFun Electronics) allowed to detect multiple tags simultaneously and to assign them to the same cow.

Each cow was provided with a halter to which five RFID tags were attached. Two positions were experimented with to optimize the cow recognition system: configuration 1) three tags on the chamfer, two tags under the jaw (three weeks, twelve cows); and configuration 2) three tags between the ears, two tags on the chamfer (four weeks, sixteen cows). Each set of tags was encoded to send a unique (low tension) frequency signal, corresponding to one of the cows (SparkFun Simultaneous RFID Tag Reader Library "Write_EPC", Arduino 1.8.19).

A UHF RFID reader antenna was placed in each of the CHS, under panel G for configuration 1, and on panel T for configuration 2 (Figure 4.1). Using the "Read_EPC" library (SparkFun Simultaneous RFID Tag Reader Library, Arduino 1.8.19), the detection range of each antenna was set to 2000 dBm to match the diameter of the hood. Upon arrival in a cubicle, tags worn by a cow were detected by the antenna. An R2R network was then used to transform the unique cow identifier to an analogue output that could be read by the datalogger. In the datalogger code, a counter was programmed to increment by one for each successful identification of a tag present in the antenna's detection range. Once the counter had reached the set value of three (corresponding to 1 minute of presence in a CHS), a MC would start. In the event that a cow moved or left the cubicle before the counter reached the pre-set value, a decrement of one was deducted from the counter for each missed reading. Eventually, the counter reached zero and other CHS were scanned again for occupancy. The implementation of this counter as a prerequisite to measurements' initialisation has allowed to limit the loss of data related to interrupted MC.

The MC always operated according to the same predefined pattern. First, the background methane concentration around the cubicle (5, Figure 4.2) was monitored for 6 minutes to differentiate the cow-specific methane production from other ambient emissions (*e.g.* herd, manure pit). The first minute of sampling corresponded to the flushing of the remaining gas in the inlet tubing and its replacement by the newly sampled gas (build-up time). The corresponding data were removed from the later analysis. Next, background methane concentrations (ppm) were measured by the FTIR for 5 minutes, with one mean reading every 21 seconds. Then, monitoring automatically switched to the hood inlets (A, B, C, Figure 4.1). Similarly to the background measurement, the first minute of data was deleted, followed by a 15 minutes monitoring of the cow's methane production. Lastly, the background methane concentration was monitored again, identically to the first MC step.

Figure 4.3. Picture of a methane flux measurement with one cow lying in the second of the four CHS units of this study (2020).

4.2.4. Head position monitoring

When monitoring breath components, information on the orientation of the head of the animal in the measurement device is essential (Hammond et al., 2016a). Muzzle position and distance to inlet have been found to be highly correlated with measured gas concentrations (Huhtanen et al., 2015b), making the filtering of data measured during inadequate head positionings key to the obtention of accurate MPR estimates. In the case of the CHS, postures in which the cows' busts were present between the rear flap (6, Figure 4.2) and the *R*, *L*, *F* and *T* panels (Figure 4.1) were classified acceptable, as they allowed the muzzle to be positioned acceptably under the extraction hood (Figure 4.4, A). Postures other than this positioning led to rearwards orientation of the snouts (Figure 4.4, B) and breath exhalation onto the background sampling points (*5*, Figure 4.2) instead of into the hood's inlets. In these cases, collected air samples corresponded to ambient air and could not be used to estimate the individual MPR.

To filter out these skewed samples, a series of nine ultrasonic sensors (**ULS**) (HC-SR04, Sparkfun) were evenly distributed over the first layer of panel *F* (3 x 3, Figure 4.1), facing the head of cows. All sensors were programmed (Arduino Uno Rev3, Arduino IDE) to propel a series of twenty-one consecutive pulses. Depending on the distance between the ULS and the

cow's bust, each pulse was converted into a higher or lower voltage, which was in turn expressed as a distance. The median distance measured by the pulses of each sensor was then retained as its true value. The ULS operated in sequence to avoid interference. Once all sensors had emitted twenty-one pulses each, only the smallest distance measured by each column was stored, and the cycle was repeated.

Figure 4.4. View of the four CHS units as simultaneously seen through camera 2 (A, left) and 1 (B, right).

4.2.5. Other sensors

Additional sensors were included in the CHS to collect complementary information. One flowmeter was placed in each of the exhaust piping to monitor the airflow going through the units (12 records per min, one mean value stored every 30 sec). This information was necessary to ensure that the correct extraction flow was maintained, and to calculate methane fluxes (equation [3]). One T&RV sensor was placed at the inlet of each polyethylene tube transporting the gas samples from the CHS's exhaust to the FTIR gas analyser. These data were used in the calculation of the molar volume of methane [1], and to convert ULS' mV output into a distance, as T and RV impact the speed at which the pulses travel through space. A measure of the ambient pressure was provided by the FTIR and used in equation [1]. Finally, two infrared cameras (DS-2CD2T63GO-I5, Hikvision) were placed above the cubicles as ground truth in the eventuality of missing information, unexpected events or results. Each camera continuously monitored two CHS, providing footages such as those visible in Figure 4.4.

4.2.6. Recovery tests

Three recovery tests were conducted at the beginning, middle, and end of the experiment (week 0, 3 and 7). They had two main objectives. First, they allowed to assess the ability of the system to recover known levels of methane production. This provided an indication on the presence of potential bias in the recovered values. Secondly, their repetition in time gave indications on the potential evolution of the CHS performance over time.

For this, two levels of methane flux were defined to correspond to the daily MPR of a low (200 gCH_4/d) and high (400 g/d) producing cow. They were injected into each of the CHS using the Artificial Reference Cow (**ARC**) created by Wu et al (2015). The ARC mimics the breathing and belching cycles of a cow, and can release defined amounts of CO_2, and CH_4. The flux of these two gases is controlled by two mass flow controllers (**MFC**, F-201CV-5KO, Bronkhorst High-Tech B.V., Ruurlo, The Netherlands), while the tidal volume is induced by compressed air pushing a piston through an aluminium cylinder. For these recovery tests, the methane MFC was connected to a 99.995% pure methane cylinder (equivaling 0.71 gCH_4/L), while the CO_2 and breathing simulations were not activated. An additional polyethylene tube (internal ⌀ = 4.35 mm) connected to the ARC outlet served as gas injection line.

For both treatments (200 and 400 gCh_4/d) and each repetition of the recovery test, the same protocol was followed (Figure 4.5). The injection line was placed directly in front of the CHS inlet B (Figure 4.1) and the injection flow set to either 0.2 L/min (≈0.14 gCH_4/min) or 0.4 L/min (≈0.28 gCH_4/min), which respectively roughly corresponds to the daily production of 200 and 400 grams (with some variation depending on the ambient conditions of the day. Background methane concentration was monitored (5, Figure 4.2) for 6 minutes (B1) before switching to the hood for 11 minutes (H), and back to the background for 6 additional minutes (B2). For the same reasons as for the MC, the first minute of each sampling was discarded as build-up time. Concentrations (in ppm) monitored over each sampling period were averaged, providing one mean ppm reading for B1, H, and B2.

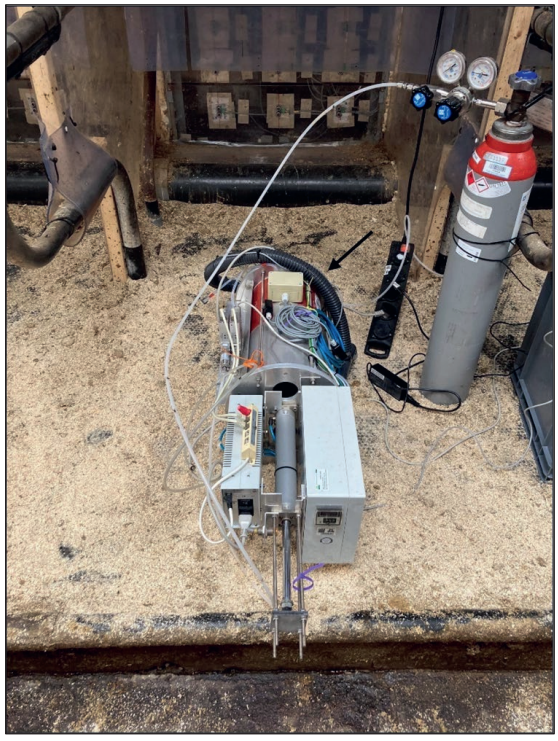

Figure 4.5. ARC and methane cylinder placed in one of the CHS before proceeding to the recovery test. During a recovery test, the outlet (black tubing, indicated by the arrow) is placed under the hood.

The conversion from the concentration readings to a daily MPR was done following the equations [eq. 4.1] to [eq. 4.3]. In a later phase, the same equations will be used to calculate the MPR of cows estimated by the CHS for each MC.

First, the methane's molar volume at the time of sampling was calculated as shown in [eq. 4.1], as the (molar) volume occupied by a gas is dependent of the ambient temperature and pressure (Charle's Law, and Gay-Lussac's Law).

Methane molar volume:

$$V_M = R \times T \div P \qquad \text{[eq. 4.1]}$$

Where V_M is the molar volume ($m^3.mol^{-1}$), R the universal gas constant ($J.mol^{-1}.K^{-1}$), T the temperature (K) and P the ambient pressure (Pa).

Consecutively, the methane concentration of the measurement (expressed as the difference between background and hood sampling) was deducted as shown in equation [eq. 4.2].

Methane concentration:

$$m = ([CH4]_H - [CH4]_B) \times 10^{-6} \times (M_{CH4} \div V_M) \quad \text{[eq. 4.2]}$$

Where m is the increase in methane concentration in the sample (kg/m^3), $[CH_4]_H$ and $[CH_4]_B$ are the methane concentrations (ppm) measured in - respectively - the hood and the background ($[CH_4]_B$ being the mean of B1 and B2 concentrations), M_{CH4} the molar mass of methane (kg.mol^{-1}) and V_M the molar volume (m^3.mol^{-1}).

For reading purposes, the methane mass was ultimately converted into a daily flux, thus providing an estimate of the MPR over 1-d [eq. 4.3].

Daily methane production rate:

$$MPR = m \div 1000 \times Q \times 24 \quad \text{[eq. 4.3]}$$

Where MPR is the methane production rate per day (g/day), m the methane concentration (kg/m^3) and Q the airflow going through the CHS (m^3/h).

4.2.7. Statistical analysis

All statistical analysis were done with R statistical software x64 4.1.0.

The ability of each RFID configuration to accurately identify cows was evaluated by comparing its outputted ID to the cow that had been identified in the videos for each MC. A true and false identification count was then made and converted into a percentage of correct identifications. The effect of cows and CHS units on the accuracy of the identifications made by the RFID was investigated with a Generalized Linear Model (**GLM**) for discrete data (Poisson regression).

Recovery rates (%) were calculated according to equation [eq. 4.4]. They provided an indication on the percentage of the methane flux that the CHS were able to recover, as well as on the presence of systematic errors between injected and recovered values.

$$\text{Recovery rate} = \frac{m_{recovered} \times 100}{m_{injected}} \quad \text{[eq. 4.4]}$$

The coefficient of variation (**CV**) was computed for each CHS and treatment to determine the magnitude of the variation between replicates.

One-way ANOVA were computed to test for the presence of significant differences in recovery rates between 1) treatments, 2) repetitions, and 3) CHS units.

An orthogonal linear regression was fitted on the recovery rates obtained across treatments to assess the proportion of variation in recovery rates that is explained by differences in the injected flux. An orthogonal regression was preferred to a standard linear regression, as recommended by the European Committee for Standardization (NEN, 2017) in cases where the uncertainty attached to each result given by the reference method (ARC) is not neglectable compared to the uncertainty of the individual results given by the alternative method (CHS). Therefore, the orthogonal linear relation between injected and recovered methane fluxes was defined as:

$$\bar{y}_i = C_0 + C_i \bar{x}_i) \qquad \text{[eq. 4.5]}$$

Where

$$C_0 = \bar{\bar{y}} - \frac{s(\bar{\bar{y}})}{s(\bar{\bar{x}})}\bar{\bar{x}}$$

$$C_i = \frac{s(\bar{\bar{y}})}{s(\bar{\bar{x}})}$$

With $\bar{\bar{y}}$ the mean injected flux, $\bar{\bar{x}}$ the mean recovered flux, and $s(\bar{\bar{y}})$ and $s(\bar{\bar{x}})$ their respective standard deviations.

It was used to calculate R^2, which indicates the percentage of variance in the response variable that is explained by the regression model. Pearson's correlation coefficient (*r*) was derived from R^2, and denotes the level of correlation between the injected and recovered methane fluxes. In addition, the root mean square error (**RMSE**) were calculated to measure the estimation errors made by the device, with smaller values reflecting a lower level of error.

Lastly, the computation of the intraclass correlation coefficient (**ICC**) provided information on the level of repeatability (and therefore reliability) between replicates. Under the mixed effect model of the absolute agreement between two judges (ARC and CHS), the ICC was calculated as (Nakagawa and Schielzeth, 2010; Sokal and Rohlf, 1995):

$$ICC = \frac{\sigma_\beta^2}{\sigma_\beta^2 + \sigma^2} \qquad \text{[eq. 4.6]}$$

Where σ_β^2 is the variance of the between-judges estimates between the replicates and $\sigma_\beta^2 + \sigma^2$ the total variance (addition of the variance of interest and the unwanted variance).

4.3. Results and discussion

4.3.1. Identification of cows using RFID

For configuration 1, a total of 155 MC were monitored, of which 96.1% were correct identifications, which was very satisfactory. However, these results were obtained for the first 2 groups of cows only, and the identification system failed as of group 3. Due to this system failure, and despite the previously satisfactory performance of the RFID, all data acquisition stopped for that group. It resulted in the implementation of configuration 2, which provided 631 MC, of which 63.1% were correct identifications. The clear cause of the failure of the first setup was not identified, and all tags were replaced each week of configuration 2 as a prevention. Still, the performance of this configuration never reached the one of the first setup. Retrospectively, it might have been preferable to keep configuration 1 in place while frequently replacing the used tags. The complementary Poisson-regressions showed that these results were not significantly different between CHS units (p = 0.165), and that only two cows led to significantly different levels of identification correctness (p cow a = 4.01e-05; p cow b = 0.004). This can be explained by their tendency to lie down leaning backwards, in which cases cows in adjacent cubicles were being detected, leading to incorrect identifications.

In general, the main source of error in identifying cows seemed to be related to the lying position of both the monitored cow and the cow(s) lying in adjacent cubicles. In some positions, the tags worn by one cow were located closely to the adjacent antenna, which could pick its signal. These cases have led to errors and misidentifications. As the lying position of the cows may not be influenced, mastering the detection range of the antennas is crucial. Unfortunately, the metal components of the cubicles seemed to affect the detection range of the antenna in an unpredictable way, making it more difficult to control.

In the tested configurations, the identification of cows using RFID did not offer a sufficiently reliable way to identify individuals. However, the identification process is of great importance as it is used to link the MPR estimates to the correct animal, information which is then used to select rations or lines of cows. Even though options are available to improve the RFID system, any electronics placed in a barn add up to the complexity of the CHS and to its maintenance. We believe that it is preferable to minimise sources of failures and errors, and that the CHS should be made as easy to use as possible, without affecting its MPR estimation accuracy. Therefore, in future studies the current cow identification system will be replaced by a camera vision recognition of coat patterns, following the work of Andrew et al (2020, 2016).

Preliminary work has already highlighted that the main challenges will be recognising mainly unicolour cows, and attributing the coat patterns of both flanks to the same cow.

4.3.2. Monitoring head pose using ULS

Unfortunately, the dataset obtained with the ULS could not be processed. Indeed, they were extremely affected by humidity and dust, the presence of which cannot be avoided in barns, even in spite of all the measures taken to do so. As a result, their distance output often resulted in unreliable data (*e.g.* distances out of the plausible range), and electronical parts required frequent replacement due to failures. Lastly, some parts of the CHS structure fell within the detection range of the ULS of the two outer columns. This sometimes lead to a detection of the CHS' side panels instead of detecting the monitored cow.

Waterproof and dustproof ULS are available on the market. Since this experiment was conducted, they have become affordable, which no longer makes cost a limitation. However, ULS sensors still require additional electronics and wiring to operate. These are not water or dustproof, add to the initial labour, and to the maintenance level once placed in the barn. For the CHS to be a practical tool, labour and maintenance levels should be minimized where possible. In addition, it proved difficult to conciliate a sufficiently large detection range with immovable within-range parts of the CHS. Therefore, it was decided not to continue with the ULS sensors to monitoring the head pose.

Currently, a computer vision algorithm is being developed to infer the head pose of the animals from the video material. Once optimised, it will be used to automatically filter out biased samples in a cheap, autonomous, and robust manner.

4.3.3. Recovery tests

Across all CHS, treatments, and repetitions, the mean recovery rate equalled 110.5% ±8.7. Treatment 1 (200 g/d) resulted in a mean recovery rate of 113.6% ±9.6 across CHS and repetitions, and treatment 2 (400 g/d) in a recovery of 107.4% ±6.6. A summary of the recovery rates is presented in Table 4.1, and a complete overview in Appendix D. Recoveries that are higher than 100% are most likely the result of the cumulative uncertainties of all sensors which's data are included in the calculations. However, it may also reflect errors in the gas sampling strategy, for example in monitoring background methane concentrations, which have

a direct impact on the estimated MPR. Various background sampling strategies should therefore be tested in the future to investigate their effect on the methane fluxes recovered by the hood.

The computation of one-way ANOVA showed that there were no significant differences in recovery rate between CHS units ($p = 0.207$), and plausibly no significant difference between in recovery rate between treatments ($p = 0.080$). These results show that the recovery performance of the CHS does not differ between measurement units nor MPR level. An additional ANOVA showed that recovery rates did not differ significantly between the repetitions ($p = 0.148$), indicating that the CHS ability to estimate MPR did not change in 7 weeks. We conclude that the MPR estimates made by the CHS (during this experiment, and within the range of 200 to 400 g CH_4/d) can be corrected for using the regression equation shown in Figure 4.6. For the longer term, conducting recovery tests at larger time intervals and over a longer timeframe (*e.g.* every month for a year) would provide essential information on the durability of the system and its estimates. In addition, the orthogonal linear regression of injected and recovered fluxes showed levels of correlation ($r = 0.988$) and explained variance ($R^2 = 0.977$) close to unity (Figure 4.6). The RMSE equated 34.95g, which is acceptable for MPR levels of 200 and 400g/day. These values reflect the satisfactory level of linear relationship between injected and recovered fluxes. The computation of the ICC yielded a value of 0.942 which shows the excellent repeatability and reliability between the methane fluxes injected by the ARC and the ones recovered by the CHS across replicates.

The results obtained in the recovery tests place the CHS as a promising tool to monitor MPR from individual cows on barn. In an upcoming step, the daily MPR estimations made by the CHS for individual cows should be compared to the ones made by a reference method (*e.g.* climate respiration chamber) for the same individuals. However, differences in estimated MPR due to biological factors resulting from the animals may be confounded with differences due to methodological factors. Therefore, the effect of biological factors and measurement errors on the accuracy of the estimate should be distinguished by performing recovery tests in addition to the actual live measurements (Gerrits et al., 2017).

Table 4.1. Mean recovery rate (%) and standard deviation per CHS unit, across treatments and repetitions.

CHS	Mean recovery rate (%)	Standard deviation (%)
1	106.95	7.75
2	113.10	7.30
3	105.62	7.17
4	116.21	9.54

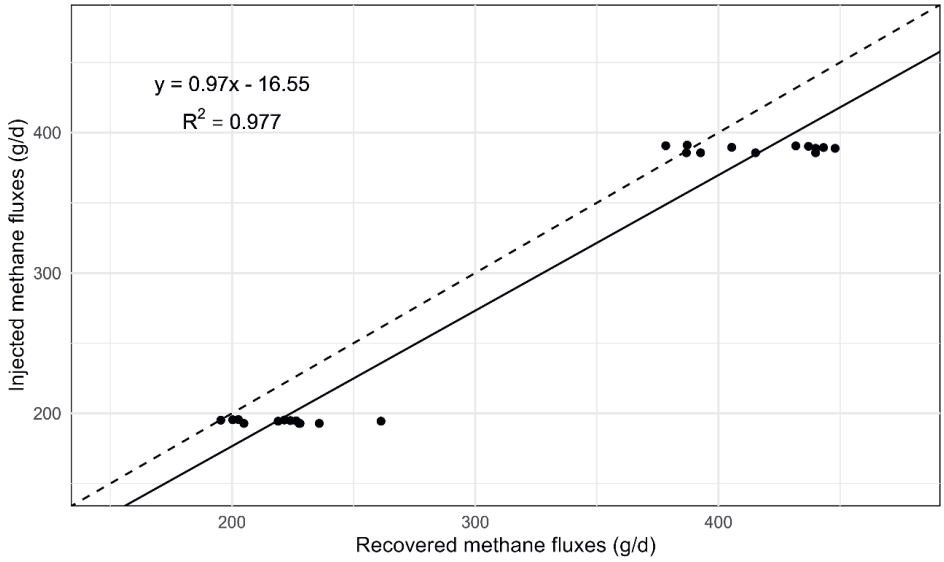

Figure 4.6. Orthogonal linear regression of the methane fluxes injected by the ARC and recovered by the four CHS, across CHS units, treatments, and repetitions (plain line). Dashed line represents x = y.

4.3.4. Further optimisation

In the upcoming phase, the sensors that were used to identify cows and monitor their head positions will both be replaced by camera vision algorithms to improve performance and reduce costs and labour. An algorithm is already being developed to infer the head pose of the animals, and the work of Andrew et al (2020, 2016) will be used as baseline to identify cows based on their coat patterns in an upcoming step. Both algorithms will be used post-hoc as their results are not needed in real time, but only to filter the data and attribute it to the right individual.

Additional attention will be given to the overall gas sampling and monitoring. Indeed, the current gas analysers which are available are globally either rather expensive, or cheaper but less accurate. Therefore, only one gas analyser could be used in this study, which resulted in a

sequential monitoring of cows with an alternating between hood and background, and a reduced data collection. However, accurate methane gas analysers are currently on their way to becoming significantly cheaper, which could allow for each CHS to be equipped with its own gas analyser within a few years. In that case, sampling strategy would be eased as continuous sampling would not require MC initiation or ending, thus simplifying the system. The monitoring of background methane concentrations could also become continuous, which would concurrently increase the accuracy of the MPR estimates.

Lastly, the portability of the CHS must be improved as its portability and adaptability to varying cubicles dimensions are simply lacking in its current design. This would allow for the system to be easily transported and implemented in multiple locations and various barn setups. Overall, we wish for the CHS to be as cheap and easy to use as possible, while maintaining the accuracy of its measurements.

4.4. Conclusions

In this study, we presented the specificities of the latest prototype of the Cubicle Hood Sampler. The head position monitoring system relying on ultrasonic sensors appeared to not be well suited for barn use. The cow identification setup using radio frequency identification first seemed promising, but resulted in unstable and unsatisfactory levels of correct identification. Therefore we have decided to replace these sensors by two camera vision algorithms. Finally, the recovery tests conducted over the range of methane production rates at which the system is expected to perform position the Cubicle Hood Sampler as a promising method for on-barn monitoring of methane production rates from individual dairy cows.

Disclosures

The authors declare no conflicts of interest financial or otherwise.

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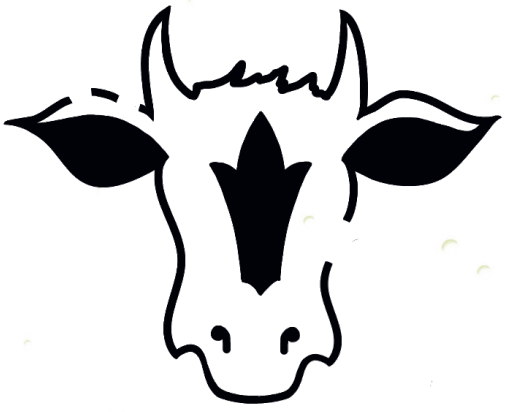

Chapter 5.

Evaluation of the cubicle hood sampler for monitoring methane production of dairy cows under barn conditions

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Highlights

- Methane production rates estimated by the current cubicle hood sampler design are biased.
- Modelling could not compensate for the biased input information.
- Ranking animals according to their methane production levels is already possible with the cubicle hood sampler, but there is room for improvement.
- Sources of bias must be identified and addressed to improve the accuracy of the estimates.
- Biases may be due to low breath recovery and its effect on background measurements.

Abstract

Monitoring methane production from individual cows is crucial for the implementation of greenhouse gas reduction strategies. However, monitoring methane production rates (**MPR**) under practical conditions remains challenging. In this study, we investigate the performance of a potential solution to this challenge.

The cubicle hood sampler (**CHS**) is a monitoring device placed in the cubicles of barns, that collects the air exhaled by cows when lying down. The methane production rates (**MPR**) of 28 dairy cows were measured by four CHS devices and compared to the levels measured by climate respiration chambers (**CRC**). A linear regression showed no strong correlation between the two sets of estimates (r=0.24). Estimations made by the CHS appeared to be biased, which cannot be corrected for in the absence of a strong linear correlation. Using Bayesian modelling, information was borrowed across cows to simulate complete methane production curves in an attempt to improve the MPR estimation accuracy. However, the model could not compensate for biased observations, and accuracy levels did not improve. An under-recovery of the breath samples by the hood is suspected. These issues must be addressed. Nevertheless, the CHS ranked cows satisfactorily, with Kendall W values of 0.625 (p = 0.201) in the original dataset, and 0.659 (p = 0.214) after using the model. Resolving the bias issue is expected to have a simultaneous positive effect on both ranking agreements. We advise to use the HMR model to borrow information across cows, and convert discrete measurements into methane production curves, as it will provide more realistic MPR estimations.

5.1. Introduction

Global anthropogenic activities contribute to the emission of greenhouse gases (**GHG**), mostly in the form of carbon dioxide (**CO_2**), methane (**CH_4**), nitrous oxide (**N_2O**), and fluorinated gases (EPA, 2012). At the current emission levels, they contribute to the enhanced greenhouse effect, which has already and unequivocally started causing climate change (IPCC, 2023), with notable effects on the environment. It is therefore urgent and essential that the GHG emissions from anthropogenic activities drastically reduce.

For their part, the global GHG emissions related to the agricultural sector (within the farm gate), represent 9 to 14% of the total share (Mbow et al., 2019), with about 6.2 ± 1.4 Gt CO_2-equivalents per year (IPCC, 2013). The non-CO_2 emissions from livestock were estimated by Herrero et al. (2016) to range between 2.0 and 3.6 Gt CO_2-equivalents per year within the farm gate, and by the FAOSTAT (2018) to have been around 4.1 ± 1.2 Gt CO_2-equivalents yearly between 2010 and 2016. Irrespective of the inclusion or exclusion of outside-the-farm-gate parameters (*e.g.* energy and land use, transportation), all references agree that cattle is the main source of global emissions from the livestock sector, with 65-77% of the share (FAO, 2021a; Mbow et al., 2019). Considering that livestock's emissions are for 40% due to methane resulting from enteric fermentation (Vonk et al., 2018), and for 9% (5% N_2O, 4% CH_4) due to manure and its management (FAO, 2014), reducing the enteric methane emissions (essentially from cattle) is one of the priority reduction pathways.

Approaches are available to reduce the enteric emissions at the animal level. In cattle, they are essentially oriented either around feeding, or genetic selection. Diet being the main driver for enteric methane formation, changes in the ration's content is the most effectively straightforward approach to reduce its production (Haque, 2018; Kebreab et al., 2010). For example, a reduction of the non-digestible fibre content (Hills et al., 2015) or an increase of the starch content (Hart et al., 2015) will lead to a diminution of the methanogenesis, and thus to the amount of enteric methane produced. Other possibilities are, for example, the increase of the share of concentrate in the provided ration (Aguerre et al., 2011; Tyrrell and Moe, 1972), or the supplementation with additives or probiotics (Knapp et al., 2014). Genetic selection, for its part, can be used to breed lines of animals that naturally and permanently produce less methane (Boadi et al., 2004). Several studies demonstrated the presence of significant variation in methane production between animals, in relation with phenotypic traits and heritability

(Breider et al., 2019; Lassen and Løvendahl, 2016). This approach has the key advantage of permanently reducing the methane production of the animals.

However, evaluating the performance of these reduction strategies requires the availability of devices that can accurately quantify the methane production rates (**MPR**) of individual cows through time. While the gold standard climate respiration chamber (**CRC**) allows a very accurate monitoring of this production rate, its costs, intrusiveness, and impracticability (cannot be used in on-barn) do not make it well suited for large scale applications. Conversely, the practical methods already available (*e.g.* GreenFeed, sniffers, use of SF_6 as a tracer gas) all present challenges with respect to measurement accuracy and throughput, animal intrusiveness, practicability, and costs (Hammond et al., 2016a; Negussie et al., 2017). Additionally, they only allow for spot sampling as they are often limited by material and labour resources, or rely on the voluntary visit of animals to the monitoring device, and therefore result in observations that are not well distributed over time and are prone to bias (Hammond et al., 2016a).

Given the non-linearity of the methane production curve, which is known to vary over time and in response to feeding (Hristov et al., 2013b; K. A. Johnson and Johnson, 1995), Wu et al. (2016) introduced the Cubicle Hood Sampler (**CHS**) as an innovative approach to monitoring MPR over significantly longer periods of time than other spot sampling methods allow. Placed in the cubicles, this device has the potential of non-intrusively monitoring the MPR from cows for up to 12-h per day, making it very promising. The design of its latest prototype was developed and presented in Chapter 4.

The aim of this study was to assess the ability of the latest CHS prototype to measure the known MPR of a set of dairy cows. To achieve this, we compared the individual MPR of 21 cows as measured by the CHS, and by the reference CRC. The correlation between the individual methane production levels measured by the two devices was studied. Additionally, ranks were computed and compared. Finally, the added value of converting discrete CHS measurements into continuous curves, by borrowing information across cows, and using a Bayesian hierarchical model (Chapter 3) was investigated.

5.2. Material and methods

5.2.1. Animals and experimental design

The two datasets (CRC and CHS) were collected during a study conducted from August to October 2020 at the animal research facilities of Wageningen University & Research (Wageningen, the Netherlands) under the Dutch Law on Animal Experiments and in accordance with EU Directive 2010/63. All experimental procedures were approved as complying with the regulations on animal experiments in vigour. The CRC experimental procedures were approved by the Central Committee of Animal Experiments (The Hague, the Netherlands; 2017.D-0079.004). The CHS procedures were classified by the Animal Welfare Officer as a non-animal experiment, as referred to in the Dutch Act on Animal Experiments.

The experiment was conducted with 28 lactating Holstein-Friesian cows (mean 2.3 ± standard deviation 0.9 lactations; 93 ± 27 days in milk, **DIM**). Cows were blocked (7 blocks of 4 cows) based on parity and dry matter intake (**DMI**), and fed a basal total mixed ration (**TMR**) throughout the entire study consisting of 41% corn silage, 32% grass silage, and 27% concentrate on a dry matter (**DM**) basis. The TMR was formulated to meet 100 and 95% of net energy for lactation and metabolizable protein requirements (CVB, 2018), respectively, for cows consuming 22 kg DM/d and producing 34 kg/d of milk containing 4.0% fat and 3.4% protein.

Each block of cows was first housed separately in a free-stall barn for 7-d to facilitate ad libitum feed intake measurement of the 4 cows within each block. The average feed intake of the block during the final 3-d of the 7-d free-stall ad libitum intake period was used to set a fixed daily feed allocation for individual cows within the block. This fixed amount (equal for all cows within a block) was fed individually during a 12-d tie stall period and a 4-d measurement period in CRC. During the additional 7-d period in which cows were housed in the CHS barn, blocks were fed collectively with the daily feed allocation defined in the first phase.

After 12-d of adaptation to movement restriction in tie stalls, cows were moved into CRC for a 4-d measurement period to facilitate determination of gaseous exchange. The design and principles of the CRC at Wageningen University (Wageningen, the Netherlands) have been described in detail by Van Gastelen et al. (2015) and Heetkamp (2015). The CRC consist of four chambers (each with a ground surface of 11.8 m^2, and a volume of 34.5 m^3) separated by airtight walls equipped with windows. During the experiment, the relative humidity and

temperature were maintained at 80.1% (± 1.2) and 10°C (± 0.1). Air renewal was maintained by continuously pumping outside air into each chamber at a ventilation rate of 43 m^3/h (± 1.1). The ambient pressure was maintained at a value of 102.9 kPa (± 7.36). Inlet and outlet air of each compartment were sampled for their CH_4, CO_2 and O_2 content to be analysed in sequence every 12 to 15 min by a series of ABB Advance Optima AO2000 analysers (ABB, Berlin, Germany). Milking and feeding occurred twice daily during the entire experiment in the CRC (0500 and 1530 h). Gas measurements during the time points when staff entered the CRC compartments for milking and feeding (maximum 30 min) were maintained in the dataset as humans do not emit significant quantities of CH_4 and because their inclusion does not affect the daily production of CH_4 (van Gastelen et al., 2017). Postprandial durations during the measurement period were calculated as the time differences from the feeding times to the occurrence of each gas sample, giving values between 0 and 13.5-h. For the purpose of the study, MPR that were not monitored over a complete 24-h period were removed from the dataset as they provided biased estimates. Thus, data from 15:30 on d 1 in the CRC until 15:30 on day 3 in the CRC was used, and represents the original CRC set (**ORS**, 9064 observations).

Afterwards, each block was moved to a free-stall barn equipped with 4 CHS units for a period of 7-d. Access to other cubicles was restricted to maximize data collection. The design and principles of the CHS have been described in Chapter 4. Essentially, they can be characterized as hoods that are placed over one of the cubicles' end and that extract the exhaled air and belches of lying animals. Measurement cycles (**MC**) are initiated on the basis of cow recognition by radio frequency identification (**RFID**) and occur in sequence. They follow a defined cycle of measuring the background methane concentrations for 6 min (**B1**) before monitoring the ones within the CHS 0for 16 min, and switching back to the background for 6 additional min (**B2**). With this setup, only one cubicle can be monitored at the time. During a cycle, methane concentrations (ppm) are measured for 21 sec by a Fourier Transform Infrared spectroscopy (**FTIR**, Gasmet Technology) and averaged over this period of time. The airflow going through the exhaust (Lambrecht Vane Anemometer 1468) as well as the temperature and humidity of the sample (Vaisala T-RV probe HMP60) are measured every 30 sec and used to calculate methane production rates (**MPR**). Cows were milked and fed twice daily around 05:00 and 15:30. No other procedures or interventions were conducted in the barn. Due to failures of the cow identification system (initiating the MC), data was only obtained for 21 of the 28 cows. This 21 cows set corresponds to the original CHS set (**OHS**, 21 cows, 621 observations).

5.2.2. Calibrations

Calibrations of the CRC and the CHS units were done with a series of recovery tests (**RT**) conducted in both devices. For the CRC, two methane RT were conducted (before and after the experiment) as detailed in Chapter 3. They resulted in a mean recovery rate of 100.8 ± 0.5% across chambers and repetitions. The CHS were calibrated three times (before, half way through, and after the experiment) as described in Chapter 4. They yielded a mean recovery of 110.5 ± 8.7% across units, treatments, and repetitions. Attention must be given to recoveries that are higher than a 100%, as they may result from the cumulative uncertainties of all sensors whose data are included in the calculations, or to errors in the gas sampling strategy (for example in monitoring background methane concentrations). Methane production rates estimated during the experiment using [eq. 4.1] to [eq. 4.3] were calibrated according to the recovery rate of each CHS unit.

5.2.3. Hierarchical Bayesian modelling of MPR

The CHS dataset is composed of discrete measurements, with sections of the postprandial methane production curve that are not systematically monitored, depending on cows' activity, farm routine, and CHS availability. Considering the non-linearity of the methane production curve (Crompton et al., 2011; van Lingen et al., 2017), we wished to approach the estimation of individual MPR as a fitted curve, which could better represent the dynamics of the methane production process than means do. For that, we used the Hierarchical Methane Rate (HMR) model presented in Chapter 3 to convert the discrete estimates into a postprandial representation of the methane production curvature. This model is a partially pooled multilevel (hierarchical) model and a Bayesian representation of a stochastic process (see Appendix C for additional explanations on Bayesian analysis). It aims at simultaneously integrating the data of multiple individuals to maximize input information and provide a better representation of the methane production curve. The HMR thus first models the grand MPR curve of that specific population, from which 3 shape parameters (β, γ, and δ) can be derived and generalized to the studied population. The scale parameter (α), for its part, is allowed to vary over cows to represent variation in methane production levels. Individual MPR curves are then derived from the estimated population curve by allowing each cow to diverge from the original curve, through the inclusion of variation in α and measurement errors. This approach has the advantage of connecting and combining information from different cows, therefore improving the accuracy of the resulting individual MPR curvature. With such models, two approaches can be taken: the

priors' distributions given to the model's parameters can either be set as weakly informative or as informative. Weakly informative priors express considerable uncertainty regarding a parameter, but are still proper probability distributions that support inference; while informative priors encode quite strong *a priori* information about a parameter, information that often stems from previous studies or independent samples (Gelman et al., 1995).

To try to maximize the positive effect HMR could have on the accuracy of the CHS estimates, we chose to opt for informative priors. These were obtained through a preceding fit using (for robustness) an independent dataset. For this, the group of 21 cows for which CHS data was obtained was split into two based on the number of observations of each cow. Half of the individuals with the most observations were retained as the CHS test set (**HTS**, 11 cows, 481 observations), while the remaining cows (10) were combined with the 7 cows that had CRC data but no CHS observations to create the CRC training set (**RTS**, 17 cows, 5616 observations). The HMR was first fitted on the RTS dataset. The posterior values of this fit then informed the hyperparameters in the prior distributions (making them informative) for the HTS fit.

5.2.4. Statistical analysis and concordance metrics

The MPR of both original sets (ORS and OHS, n = 21) were averaged per cow and over all days yielding a single daily MPR estimate (g/d) per animal. The MPR of the HTS were deduced for each individual cow (n = 11) from the computation of the area under the cow's individual MPR curve. These areas under the curve (**AUC**) were computed according to Chapter 3 and divided by the time fraction over which they were computed (duration between two feed allocations). They yielded a single daily MPR value per cow in g/d. Data from RTS was not further used as it corresponds to a different set of cows than HTS. This set was only used to extract the parameter values that were needed for the HTS model fit.

Mean daily methane production rates (g/d) were calculated for each set with their standard deviation (sd) and coefficient of variation. A complementary autocorrelation analysis was conducted to assess the (potential) levels of autocorrelation between the MPR observations (lag of 1) collected through time, by both devices and for each cow. Each CRC observations were separated by a 10 min timespan, while time intervals between CHS records were more variable, but always larger than in the CRC. Autocorrelations are included between -1 (perfect negative autocorrelation), and 1 (perfect positive autocorrelation. A value of 0 shows no autocorrelation

between observations (Kendall, 1948; Mann, 1945). A linear regression of the individual MPR estimated by the CRC on the MPR estimated by the CHS was performed to test for any potential bias in MPR estimation by the CHS. This was first done with ORS against OHS to provide indications on how well does the system itself performs (without using the model). Then, the 11 cows subsets of ORS and OHS were regressed against each other to give a direct comparison point for the regression of the ORS subset against HTS. The latter was used to assess the added value of HMR. These regressions were used to calculate R^2, which indicates the percentage of variance in the response variable that is explained by the regression model. Pearson's correlation coefficient (r) was derived from R^2, and denotes the level of correlation between the rates monitored by the two systems. In addition, the Root Mean Square Errors (**RMSE**) were calculated to measure the estimation errors made by the CHS, with smaller values reflecting a lower level of error.

Lastly, the ability of the CHS to rank animals based on their MPR levels was investigated with Kendall's coefficient of concordance (Kendall's W) (Kendall and Smith, 1939). This non-parametric statistic measures the agreement between the ranks attributed to each individual cow based on its MPR estimated by the CRC versus its CHS one. A value included between 0 (no concordance) and 1 (absolute concordance) was obtained for the overall accordance between all ranks. Ranks obtained with the OHS, the OHS subset, and the HTS were compared to the ORS ones.

5.2.5. Software and code

The HMR model was coded in R (x64 4.2.3.) using the public libraries *Bolstad2* (Bolstad, 2010), *brms* (Bürkner, 2017), and *Rcpp* (Eddelbuettel and Francois, 2011). All analysis were computed in R (x64 4.2.3.).

5.3. Results and discussion

5.3.1. Model fit

Fitting the HMR model with RTS yielded a population curve (Figure 5.1) whose parameters took the posterior values $\alpha = 254.82 \pm 9.66$, $\beta = 2.08 \pm 0.17$, $\gamma = 2.75 \pm 0.13$, and $\delta = 0.0375 \pm 0.0007$. These values informed the location and spread of the prior distributions for the HTS fit, except for the standard deviation of α that was increased from 9.66 to 100 to enable a larger

variation in scale between cows, and better reflect their variability. It yielded a population curve (Figure 5.2) and new posterior parameters values $\alpha = 154.86 \pm 13.87$, $\beta = 1.94 \pm 0.17$, $\gamma = 2.83 \pm 0.13$, and $\delta = 0.0377 \pm 0.0007$. The increase in credible interval observed for the HTS fit reflects the lower prediction certainty of the HTS model fit that is linked to the lower number of observations available in this set.

Individual methane production curves were also derived from the fit, an example of which is shown in Figure 5.4 (see Appendix H for the other individual curves). In comparison with the CRC dataset of the same cow (Figure 5.3), it appears clearly that the CHS collects significantly lower numbers of observations, and that the peak of the methane production curve (reached 30 to 140 min after feeding) is not captured by the device. Variability between the individual estimates of MPR also appears to be significantly higher in the CHS (spread of values visible in Figure 5.4), which can be due to measurement errors themselves (hood and background), or to varying airflows and cow postures both around and within the device.

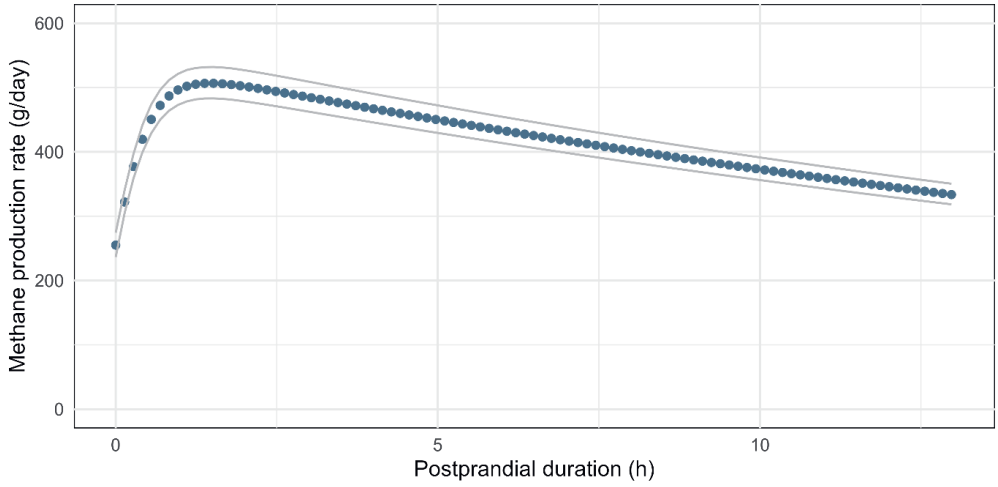

Figure 5.1. Population curve of the postprandial methane production rates obtained with the climate respiration chamber training set (RTS). Blue dots represent the MPR estimated at each time fraction of the postprandial duration. Plain grey lines represent the 95% credible interval.

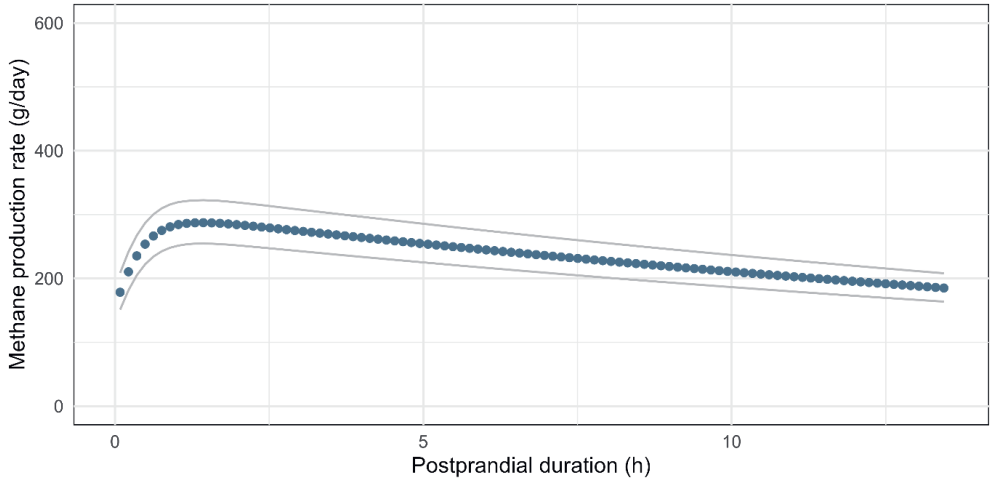

Figure 5.2. Population curve of the postprandial methane production rates (MPR) obtained with the cubicle hood sampler test set (HTS). Blue dots represent the MPR estimated at each time fraction of the postprandial duration. Plain grey lines represent the 95% credible interval.

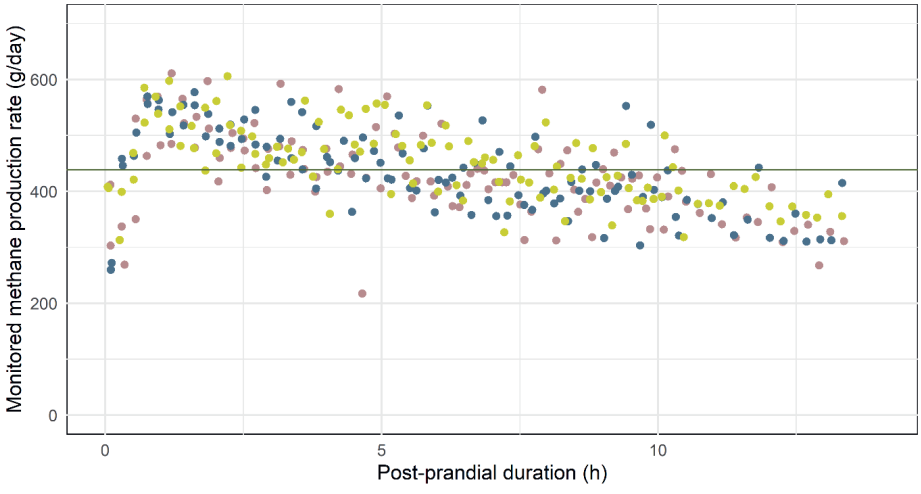

Figure 5.3. Example of the postprandial methane production rates (MPR) estimated by the climate respiration chamber (CRC) for one cow (ORS). Each colour represents values monitored over the same day of measurement (Day 1, Day 2, Day 3). The plain horizontal line represents the mean methane production rate obtained for this cow (g/d). This figure illustrates the high throughput of CRC.

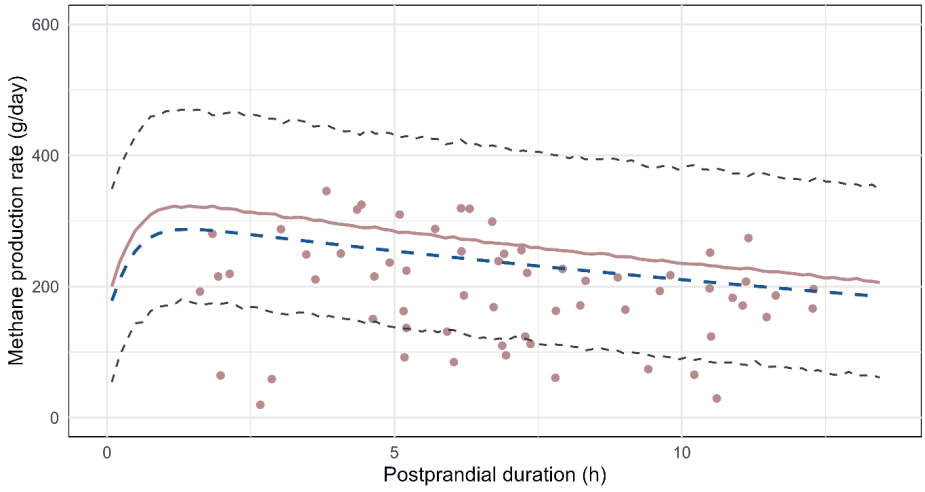

Figure 5.4. Example of the postprandial methane production curve obtained for one cow by fitting the hierarchical methane rate (HMR) model with cubicle hood sampler (CHS) data (CHS test set: HTS). Each pink dot represents the methane production rate estimated by the CHS over one measurement cycle. The dashed blue line shows the population curve obtained with the HMR fit, while the plain pink line shows the methane production curve of the individual cow of interest. Dashed grey lines represent the 95% credible intervals.

5.3.2. Absolute MPR estimates

The mean MPR obtained with the CHS (OHS, OHS subset, and HTS) appeared to be considerably lower than the levels measured by the CRC (ORS) (Table 5.1). This underestimation appears to be consistent, both across animals and through the methane production curve. Absolute levels of variation in estimated MPR between cows (sd) did not vary much across analysed CRC and CHS (OHS and HTS) datasets. However, relatively to their respective mean, they led to coefficients of variations that are about twice higher for the CHS than for the CRC. These higher levels of variability in estimates made by the CHS were expected, as they result from the known sources of errors that are associated with complex barn environments (*e.g.* complex airflows, challenging background sampling, presence of surrounding cows etc.). Additionally, the level of autocorrelations between ORS measurements yielded a mean value of 0.388 ± 0.148 between measurements within cows, showing the positive autocorrelation that is present between two consecutive estimates made by the CRC, thus reducing the overall CV. Conversely, the OHS measurements appeared to have low levels of autocorrelation, with a mean value of -0.003 ± 0.268 (Appendix E). These results can be

explained by the shorter time interval between two observations made by the CRC, and by the larger and more variable intervals observed in the CHS.

Table 5.1. Mean methane production rate (MPR, g/d) of all datasets. The original sets include the original respiration chamber set (ORS), and the original cubicle hood sampler set (OHS). The test sets include a subset of the ORS (ORS subset), a subset of the OHS (OHS subset), and the cubicle hood sampler training set (HTS). The number of cows (n) included, together with the mean MPR (g/d) of each dataset are presented with their standard deviation (sd, g/d), and coefficient of variation (CV, %).

	Original sets		Test sets		
	ORS	OHS	ORS subset	OHS subset	HTS
n cows	21	21	11	11	11
Mean (g/d)	415.4	242.9	403.3	236.6	234.3
sd (g/d)	36.9	44.5	32.8	36.1	34.5
cv (%)	8.9	18.3	8.1	15.3	14.7

The regression of OHS, OHS subset, and HTS on ORC yielded three regression lines. The OHS regression (Figure 5.5) resulted in the values $R^2 = 0.06$, $r = 0.24$, and RMSE = 179.8. The OHS subset regression (Figure 5.6) provided the values $R^2 = 0.09$, $r = 0.30$, and RMSE = 171.7, while the HTS one (Figure 5.6) yielded $R^2 = 0.05$, $r = 0.22$, and RMSE = 174.2. In all cases, the r values reflect the poor strength of the relationship between the MPR estimated by the CRC and the CHS. The R^2 values show the low amount of variance in MPR measured by the CRC that can be related to the CHS data. Lastly, the high RMSE values show that the CHS cannot predict the CRC levels accurately. In the absence of a strong, linear relationship between the MPR levels estimated by the CRC and the CHS, the equations of the linear regressions shown in Figure 5.5 and Figure 5.6 are not suited to be used as calibration lines. Methane production rates estimated by the CHS can therefore not be corrected for this way.

Additionally, fitting the HMR model with CHS data (HTS) did not improve the concordance metrics' values. Indeed, the model can be used to convert discrete measurements into a postprandial production curve (which is a better representation of this biological process), but it cannot compensate for estimates that are biased in the first place.

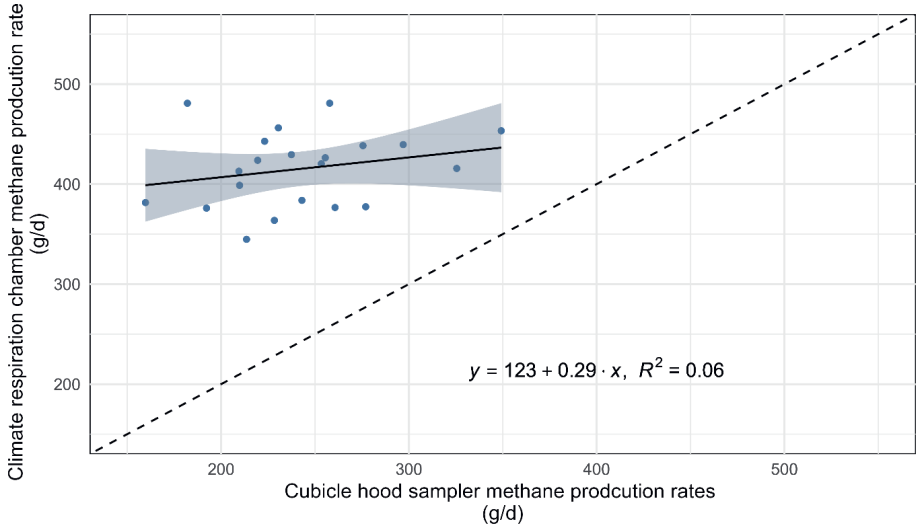

Figure 5.5. Linear regression of the individual methane production rates (MPR) estimated by the climate respiration chamber (CRC: ORS) and the cubicle hood sampler (CHS: OHS). Each point represents the average MPR of a cow as estimated by both devices, completed by the (plain) regression line over all estimates. The dashed line symbolizes x = y.

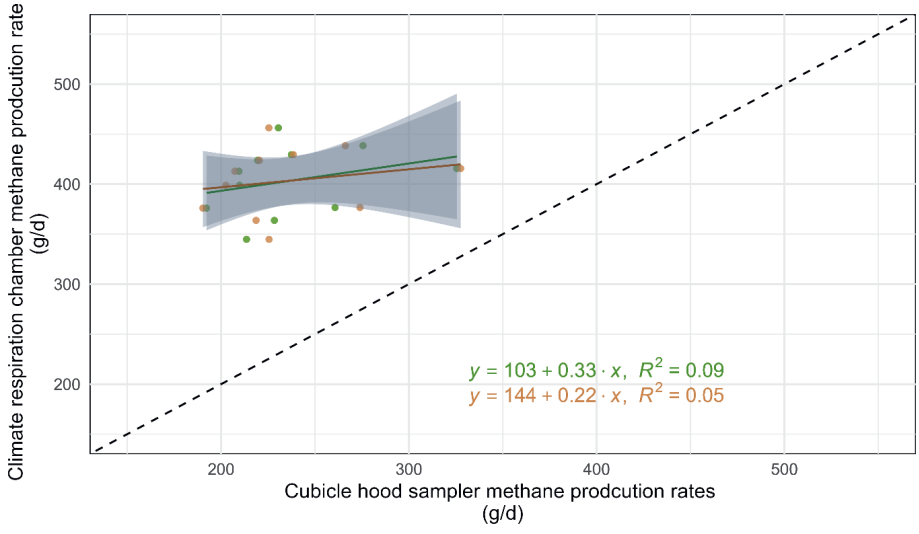

Figure 5.6. Linear regressions of the individual methane production rates estimated by the climate respiration chamber (CRC: ORS subset) against the ones estimated by the cubicle hood sampler in the original subset (CHS: OHS subset) in green, and in the test set (CHS: HTS) in brown. Each point represents the average MPR of a cow as estimated by both CRC and CHS (OHS subset: green; HTS: brown), completed by the (plain) regression line of each set of estimates. The dashed line symbolizes x = y.

5.3.3. Ranking of individual MPR

The computation of Kendall's W rankings yielded a value of W = 0.625 (p = 0.201) for the OHS (Figure 5.7), of W = 0.736 (p = 0.142) for the OHS subset (Figure 5.8), and of W = 0.659 (p = 0.214) for the HTS (Figure 5.9). These W values show that the CHS allows to rank cows reasonably well, but that errors in ranking are still being made. Selecting animals on these values must therefore be done with care. The ranking agreement obtained with the HTS appeared slightly higher than the one obtained for OHS, but lower than the one of the OHS subset. This shows that the higher ranking agreements are due to the smaller sample size (11 versus 21), and therefore the smaller chance of errors.

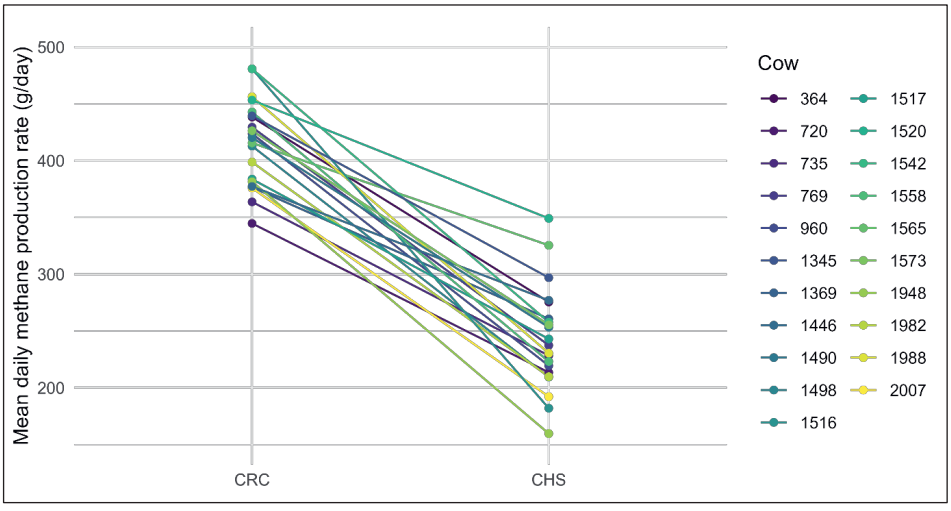

Figure 5.7. Ranks based on methane production levels estimated by the climate respiration chamber (CRC: ORS) and by the cubicle hood sampler (CHS: OHS) for twenty-one cows.

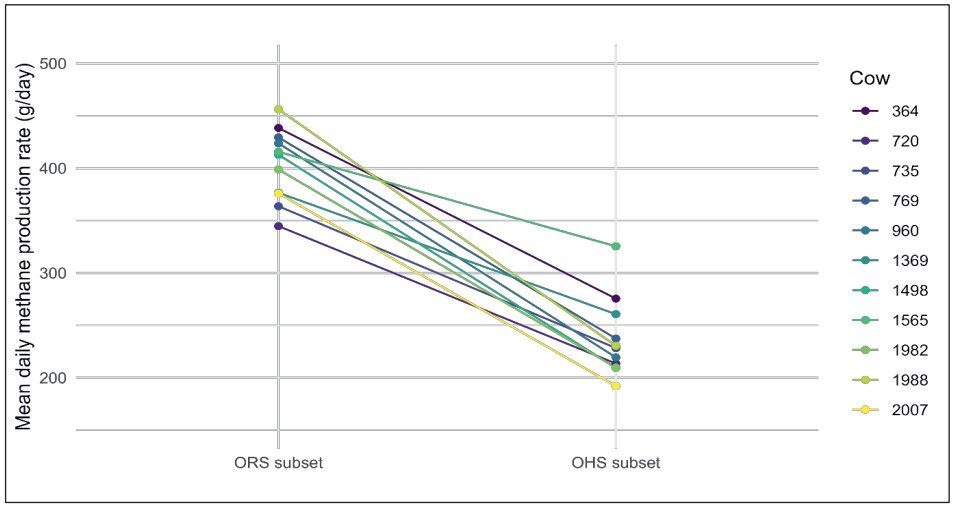

Figure 5.8. Ranks based on methane production levels estimated by the climate respiration chamber (CRC: ORS subset) and by the cubicle hood sampler (CHS: OHS) for eleven cows.

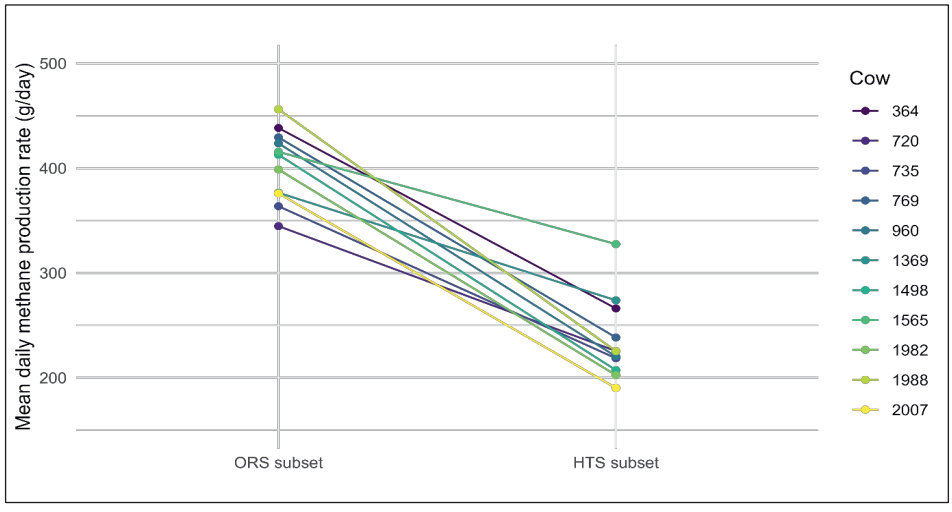

Figure 5.9. Ranks based on methane production levels estimated by the climate respiration chamber (CRC: ORS subset) and by the cubicle hood sampler after use of the hierarchical methane rate model (CHS: HTS).

5.3.4. General discussion and implications

In its current state and with the quantity of data that was collected, the CHS - with or without using HMR - cannot be used to accurately estimate the MPR of individual cows. It consistently

measured significantly lower MPR than the CRC did, and did not show a sufficiently strong linear relationship that could have allowed for its MPR estimates to be corrected for. In its current state, the CHS should not be used in experiments requiring accurate estimations of individual MPR, such as studies investigating the effect of feed or additives on methane production. The reasons behind the persistent measurement bias that have been highlighted in this work must first be identified and addressed. As measurement bias and underestimation appeared to be persistent, we hypothesise that they are essentially due to a poor overall recovery of the breath samples as such. Indeed, the RT that were conducted provided insights on the good performance of the system itself (ducting, fan, gas analyser), as injected and recovered methane fluxed were well (and positively) correlated. However, during the RT, the positioning of the injection line directly in the inlet may not have been fully representative of the actual experimental conditions, nor be indicative of the system's ability to recover a breath sample in the presence of a cow. A smoke test was complementarily carried out to get an idea of the system's ability to suck air into the inlet, and to detect potentially unwanted airflows. For this, smoke was released with a smoking machine at various distances and angles from the inlet points (roughly 10 to 40 cm, 0 to 180°). This showed that the smoke was satisfactorily sucked into the hood, and this even when the smoking machine was oriented backwards from the inlets. However, the amount of smoke that was recovered by the CHS in varying release positioning could not be quantified. Moreover, smoke alone cannot simulate the complex air dynamics that occur in the presence of animals (*e.g.* exhalations blowing air in a direction, obstruction due to cows' bodies, creation of heat and humidity) and at varying dates, times of the day, and weather conditions. Indeed, methane concentrations have already been shown to vary around a previous prototype of the CHS, both temporarily and spatially (Wu et al., 2016). These variations were essentially attributed to changes in airflow patterns and air speed in the barn. Additionally, this study highlighted the significant impact of background measurements on MPR estimates, with the variation in background methane concentrations strongly influencing the overall CV of the measurements made by the hood. This connects to our second hypothesis, which is that measurement errors made for the background sampling might strongly influence the resulting MPR estimates. As shown in equation [eq. 4.2], background methane concentrations are subtracted from the ones that are measured by the hood. Therefore, errors made in the background values will bias the overall MPR estimate. Simultaneously, a poor recovery of the breath sample by the hood' inlets signifies that the remaining breath is (at least partially) redirected towards the background sampling points. This means that not only the hood itself

will under-record methane flux, but that background concentrations will be overestimated, thus amplifying the underestimation effect of the hood measurement.

To address the aforementioned issues, and in an attempt to resolving the current measurement bias, we suggest to assess the recovery performance of both hood and background samplings. This could for example be assessed with a tracer gas method (*e.g.* SF_6), in which cows are equipped with a permeation tube releasing SF_6 at known release rate (Grainger et al., 2007; Johnson et al., 1994b). This gas only being naturally present in barns at low concentrations, the SF_6 concentrations that would be measured by the CHS hood and background sampling points would thus be very indicative of their respective recovery. If it confirms the low recovery of the hood, the suction could for example be increased, by increasing fan speed, or redesigning the system to maximise under-pressure.

At a later stage, accuracy of the estimates could be fine-tuned, for example by increasing the number and frequency of samples taken by equipping each CHS with its own gas analyser, enabling multiple cows to be monitored simultaneously instead of rotating measurement across cows with a single analyser. Linking methane and carbon dioxide measurements could also be done, as both production levels have been shown to be correlated (Madsen et al., 2010).

We also advise to keep using the HMR model for estimating MPR in future setups to convert the discrete observations into a continuous methane production curve and calculate daily MPR as an AUC. The HMR model did not improve the accuracy of the MPR estimates here, which is only due to the biased estimates that were used to fit it with. The ability of the HMR model to correctly fit and replicate the CRC datasets of 28 cows has already been proven (Chapter 3). By allowing for the borrowing of information across cows, the HMR model should be particularly valuable with datasets that lack information in parts of the curve (scarce measurements), and to convert spot sampling observations into postprandial methane production curve. Using it would therefore present a better representation of this dynamic process, and provide more realistic estimates of MPR.

For now, it is still possible to use the CHS to rank animals. The Kendall W values obtained for ranking showed that the CHS allows to rank cows reasonably well, with or without making use of the HMR model. Depending on the relative level of raking accuracy that are wished for in (genetic) selection studies, the CHS can be used as an alternative system to rank animals in practical settings. In the future, we expect that resolving the measurement bias issue will simultaneously lead to higher accuracy in ranking.

5.4. Conclusions

In its current state, the cubicle hood sampler (CHS) cannot measure individual methane production rates (MPR) from cows accurately as its estimates are biased in a way that currently cannot be corrected for. It can therefore not be used in experiments requiring high measurement accuracy, such as nutrition studies. However, it can be used to rank and (genetically) select animals on the basis of their MPR. Using hierarchical Bayesian model did - here - not improve the accuracy of the MPR estimates, as the model cannot compensate for biased data. We therefore suggest to address this crucial bias issue, notably by investigating the ability of the hood to recover breath samples. Simultaneously, the presence of potential errors in background sampling should be investigated, and connected to the hood measurements, especially in the eventuality of a poor hood recovery. After addressing these issues, we advise to keep using the HMR model to convert discrete measurements into a methane production curve, which both provides a better representation this process, and a more realistic estimation of MPR by accounting for variability between animals. Lastly, it should allow to deal with smaller number of observations per cow, by enabling the borrowing of information between animals.

Disclosures

The authors declare no conflicts of interest financial or otherwise.

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Chapter 6.

General discussion

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6.1. Key findings and conclusions

The research work conducted in this thesis allowed to evaluate the performance of two sets of devices that aim at monitoring the individual enteric methane production of - respectively - sheep and cows in practical conditions. Complementarily, a model was developed, and its ability to simulate the postprandial methane production curve of both a population of cows and its individuals was investigated.

In **Chapter 2**, a study was conducted to assess the ability of a set of ten portable accumulation chambers (**PAC**) units with the same design to accurately monitor the methane production rate (**MPR**) of sheep and to rank animals according to these levels. This work did not involve live animals so that the performance of the chambers could be evaluated in the absence of extraneous, confounding factors. A series of tests were thus conducted using a mass flow controller (**MFC**): a leakage test to test chambers for potential leakages; a ring test to evaluate the ability of all chambers to repeatedly record the same methane mass; and a range test to investigate the MPR estimation accuracy of two chambers at varying masses. The leakage test showed that two out of ten chambers were leaking, bringing to light the necessity for regular leak testing. The ring test demonstrated that the remaining eight chambers could consistently and repeatedly record the same methane mass, with a repeatability above 0.99 and low coefficient of variation (0.0 - 2.4%) between replicates. Furthermore, the correlation between released and recorded CH_4 masses was strong and positive ($r > 0.99$). These PAC are thus well suited for investigating relative differences between individuals. However, the measured recovery rates of masses injected in the range test showed that PAC (as used here) are not well suited for applications investigating absolute methane production rates at high accuracy levels. While there were no significant differences in recovery rate between PAC, there was however a significant shift in recovery over the range of injected masses, decreasing from 74.5% to 67.6%. If recovery tests are regularly conducted across the measurement range, they could be used to generate a calibration factor to correct for the inaccuracy. Nonetheless, we suggest thorough calibration of the gas analyser, which was not done in this test due to practical limitations. If calibrated at different humidity levels and over the concentration range at which it is expected to perform, both the shift and the under-recovering might be explained. Overall, the approach taken in this study proved to be a simple, efficient, and reproducible manner to calibrate PAC units, which could offer a way to homogenize calibration of PAC units across experiments and research groups.

Chapter 3 introduced a newly developed hierarchical Bayesian stochastic model to model postprandial methane production curves in dairy cows. The so-called Hierarchical Methane Rate (**HMR**) model is a transformation of the equation proposed by Crompton et al. (2011) into a Bayesian representation of a stochastic process that aims to simultaneously integrate the data of multiple individuals to model the grand MPR curve of that specific population under their specific housing and management conditions. This model allows the computation of an estimated population curve, from which three shape parameters can be derived and generalized to the studied population (partially pooled), whilst the scale parameter is allowed to vary over cows (hierarchical). The HMR model was fitted with climate respiration chamber (**CRC**) data of twenty-eight lactating dairy cows. The mean MPR and inter-cow variability predicted by the HMR model appeared in agreement with the levels monitored by the CRC (CRC = 416.7 ± 36.2 g/d, HMR = 407.2 ± 35.0 g/d). The linear regression of the observed and predicted individual MPR yielded a Pearson's correlation coefficient *r* of 0.997 showing a strong and nearly perfectly linear correlation. The low RMSE value of 10.0 g/d (equal to 2.4% of the observed mean) reflected the high prediction accuracy of the model. Complementarily, a posterior predictive check (**PPC**) was used to replicate data under the fitted model (1000 draws), which showed that the model could reproduce the observed data well, therefore demonstrating that it is a good representation of the biological process of interest. Finally, its comparison in a leave-one-out cross validation against the fully pooled equivalent of the model (all parameters pooled over cows) showed that the partially pooled model (HMR) can better estimate the individual MPR of the studied individuals (expected log pointwise predictive density difference of 1652.9 in favour of HMR, standard error = 69.3). Overall, the ability of HMR to predict the MPR of individual cows was shown.

In **Chapter 4**, the latest prototype of the Cubicle Hood Sampler (**CHS**) was presented, which is a device aiming at monitoring the MPR of individual dairy cows. Its design and specifications were fully shared. Preliminary results of two detection systems included in the setup were presented. First, the cow identification one, based on radio frequency identification (**RFID**), was tested in two different setups. Configuration 1 yielded 96.1% of correct identifications (monitored cow ID matching the cow present in CHS) but ended up failing, and configuration 2 reached 63.1%. With the exception of two out of twenty-one cows, identification results were not significantly different between animals nor CHS units. In general, the main source of error in identifying cows seemed to be related to the lying position of both the monitored cow and the cow(s) lying in adjacent cubicles. In the tested

configurations, identifying cows using RFID did not yield sufficiently reliable results. Secondly, the results obtained with ultrasonic sensors (**ULS**) to monitor the head position of the cows could not be processed as the sensors were too sensitive to humidity and dust, leading to recurrent failures. As a replacement, work on a computer vision algorithm has been done to infer the head pose of the animals from video material. Once optimised, it will be used to automatically filter out biased samples in a cheap, autonomous, and robust manner. Lastly, recovery tests were conducted to evaluate the ability of the system to record methane fluxes. Across the four CHS units, the two treatments (200 and 400 gCH_4/d), and the three repetitions, the mean recovery rate equalled 110.5 ± 8.7%. There were no significant differences in recovery rate between CHS units (p = 0.207), and plausibly no significant difference in recovery rate between treatments (p = 0.080). However, recovery rates that are higher than 100% are most likely the result of the cumulative uncertainties of all sensors whose data are included in the calculations. Part of this uncertainty can be due to bias in the gas sampling strategy, for example by monitoring methane concentrations in the background air that are lower than those in the air actually entering the hood. In any case, the recovery tests as conducted here consist in a calibration of the system itself freely of the extraneous factors induced by the presence of cows. They might however not be fully representative of the ability of the CHS to recover the breath samples of cows, and the performance of the device must therefore also be tested in the presence of animals.

Lastly, **Chapter 5** presents the results of the evaluation study of the CHS for monitoring the methane production of dairy cows under barn conditions. The MPR of 21 cows were subsequentially estimated by the CHS and by CRC. Direct comparison of the estimated levels showed that the CHS measured (roughly) mean MPR twice as low (CHS = 242.9 g/d, CRC = 415.4 g/d), with levels of variation between cows twice as high (CHS coefficient of variation (**CV**) = 18.3%, CRC CV = 8.9%). The linear regression of the monitored MPR yielded a Pearson's r value of 0.24 and a root mean square error (**RMSE**) of 179.8. Autocorrelation was detected between the CRC measurements of each cow (0.39 ± 0.15), but not between the ones of the CHS (-0.00 ± 0.27) as they were spread further apart in time. Subsequently, the HMR model presented in Chapter 3 was used to fit the CHS data of the eleven cows with the most observations (n_{obs} >30) using informative priors based on the posterior obtained in a previous, independent fit. The MPR levels obtained for these eleven cows were 234.3 ± 34.5 g/d, versus 236.6 ± 36.1 g/d estimated by the CHS without the fit, and 403.3 ± 32.8 g/d by the CRC. The linear regression of CRC and fitted CHS values yielded a r of 0.22 and a RMSE of 174.2, while

the non-fitted CHS data resulted in a *r* of 0.30 and a RMSE of 171.7. The MPR ranks computed in the Kendall W test resulted in very similar values, with W = 0.625 for the twenty-one cows set, 0.736 for the eleven cows subset, and 0.659 for the fitted data. Overall, the analysis conducted in this chapter showed that there is a consistent bias in the estimations of MPR made by the CHS. Using the HMR model could not compensate for this bias. As a result, the CHS (in its current design) should not be used to estimate absolute methane production rates until this (consistent bias) issue is resolved. It may however be used (with care) to rank cows, or classify them as being above or below the mean MPR level of their own population.

Overall, this work has brought to light the complexity of monitoring enteric methane production rates in practice and at individual level. Various difficulties were encountered, notably in terms of frequency and number of measurements (in connection with the general non-linearity of the postprandial methane production curve), breath sample recovery, background concentration measurements, calibrations, and overall data analysis.

6.2. From ideal situation…

Climate respiration chambers are ideal for measuring gaseous exchanges in ruminants. By holding animals in airtight compartments, they allow to clearly differentiate gas consumption and production of the studied animals from the ones of the outer environment (background). Inlet and outlet of air are entirely controllable, making dilutions minimal. Airflows are precisely controlled and used to obtain a homogeneous climate in the chamber and a good mixing of the gas components of the air, ensuring the representativeness of the air samples being analysed. Part of the outlet air is generally recirculated into the room to increase gas concentrations and therewith the measurement accuracy. However, sufficient levels of oxygen should be maintained, and CO_2 concentrations of about 1% (or 10000 ppm) in the chamber are recommended not to be exceeded (Lighton, 2018). As a rule of thumb, circulation rates should be equal to at least once the total volume of the chamber each minute to ensure good mixing. In the case of a 35 m^3 CRC, this corresponds to a minimal circulation rate of 2100 m^3/h (Heetkamp et al., 2015).

Gas analysis is usually done alternatively between chambers using a multiplexer. A series of air samples are thus collected at each chamber inlet's and outlet's and transported to a gas analyser according to the defined sampling scheme. In some designs, the samples can be cooled down for the water vapor to condense (dew point), allowing for the interference of water vapour to be equal in all samples so that it can be neglected (Heetkamp et al., 2015). As an indication,

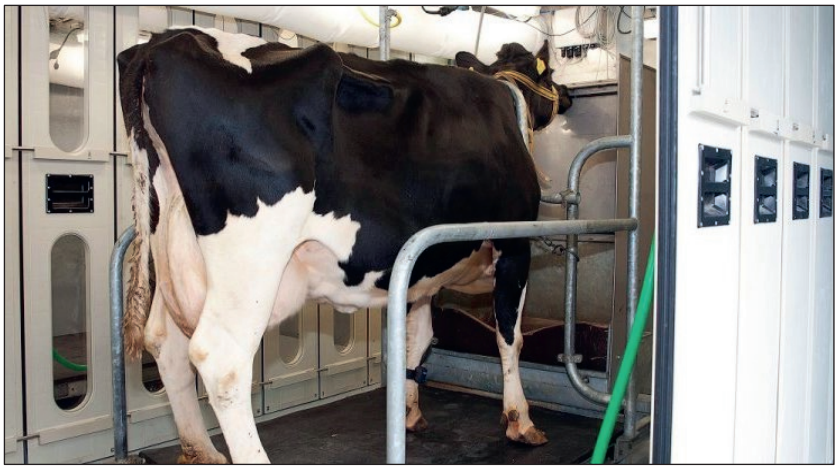
the data used for the CRC study reported in Chapter 3 corresponds to an alternating sampling between chambers, whose gas concentrations in CO_2, CH_4 and O_2 were measured over 180 sec (per chamber) every 12 to 15 min by an Advance Optima modular system (ABB Ltd.). In addition to the estimation of CO_2, CH_4 production and O_2 consumption, this information can be used to derive other indicators, for example to calculate heat production (Alferink et al., 2015). Zero and span concentrations from different gas mixtures can be used to check and adjust the zero and span point deviation of the gas analyser. In Wageningen, this is done at least once daily, and is complemented by regular full system calibrations (usually before and/or after an experiment, without animals) to ensure that high measurement accuracy is maintained.

The internal air pressure of the chambers can be adjusted as well. The units can be made hypobaric (under-pressurized) or hyperbaric (over-pressurized) depending on the study design, with recommended pressure differences of 50 to 100 hPa between rooms and outer environment. Using pressure information, leaks can be monitored and quantified to ensure that they remain within the tolerance level. In addition, the temperature and relative humidity levels of the chamber's air can be controlled to obtain the desired climate (Heetkamp et al., 2015).

Besides offering a highly controllable environment, CRC also permit a wide range of information to be obtained about the animal being studied. For example, body weight can be measured by a weighing scale that is directly placed under the animal (Labussière et al., 2015). Feed intake is controlled and monitored as rations are fed individually and eaten quantities (and refusals) are evaluated by a scale placed under the feeding bin. Even the excreta of the animals can be collected and analysed, for example when investigating the total energy balance (Heetkamp et al., 2015). In addition, CRC also possess the advantage of not being influenced by animal positioning or the presence of other animals. The head position of an animal within a chamber does not impact gas recovery as inner air is well mixed, and the absence of other animals creates no complications in terms of background or breath air sampling.

However, some downsides come with using CRC. First, the confined space they offer constrains animals movements and behaviour. In addition, the isolating nature of the CRC has been demonstrated to induce stress, leading to decreases in dry matter intake, a variable that is directly correlated with methane production (Llonch et al., 2016). Respiration chambers are also very costly, labour intensive, and do not allow the screening of animals at large scale (Lassen and Difford, 2020). Therefore, a range of other methods are being developed so that enteric methane production can be individually monitored in practice, at large scale, and without interfering with animals and farmer routines.

A



B

C)

Figure 6.1. Game "spot the differences" between cows monitored for enteric methane production with A) a climate respiration chamber, B) a GreenFeed emission monitoring, and C) a tracer gas. Credits: A) Wageningen University and Research, B) DairyNZ and University of Waikato, C) University of Kiel.

6.3. … to practical problems

As observed in the literature (Chapter1) as well as in the studies conducted in Chapters 2, 4, and 5, the practical monitoring of enteric methane production is associated with restrictions on cow handling that are imposed by management requirements, and that create several additional sources of uncertainty in the monitoring of individual animals. Compared to the gold standard CRC, these extra sources of uncertainty lead to practical measurement approaches with lower and often unsatisfactory measurement accuracies. They have been identified as corresponding to four main sources of errors: the ones related to breath recovery, those occurring in background air monitoring, the issues arising from whole-system recovery and calibrations, and finally the ones connected to the overall sampling strategy and data handling. These uncertainties must be addressed so that MPR estimation accuracy may be improved.

6.3.1. Breath recovery

First of all, errors can arise from the recovery of the breath sample itself. For measurement systems operating in open or semi-open environments (like the ones shown in Figure 6.1 B and C), there is a chance that the breath sample is not recovered by the monitoring device sufficiently well. In this case, the methane mass contained in the breath of the animal is underestimated, leading to a subsequent underestimation of MPR.

This could explain the weak correlation between the MPR estimated by the CRC and the CHS that was observed in Chapter 5. The mean recovery rates obtained in Chapter 4 for the four CHS units across the two treatments (200 and 400 gCH4/d injected with a MFC) and three repetitions did not show this, but it may be because these tests were not fully representative of the actual experimental conditions, nor were indicative of the system's ability to recover breath samples in the presence of animals. In a design such as the one of the CHS, an under-recovery of the breath is particularly problematic as it may simultaneously lead to a second drawback. The breath air that was not captured by the hood will be drawn back onto the background sampling points. Consequently, background methane concentrations will most likely be overestimated. As these concentrations are subtracted from the levels monitored in the breath samples [eq. 4.2], MPR will be underestimated even more.

Most likely, this could be due to an underperforming suction within the hood. This can be the result of an insufficient airflow going through the system, and of a poor (or non-existent) under-

pressure effect under the hood. To overcome this, the airflow can easily be adjusted by increasing the flow rate of the fan. However, preliminary studies have shown that airflows between 120 and 200 m^3/h are optimal for the CHS. Indeed, flow rates of less than 120 m^3/h resulted in injected flux recoveries lower than 100%, while airflows rates of 120-200 m^3/h achieved recoveries that were not significantly different from 100% (Wu, 2016). Higher airflows, on the contrary, have not been experimented with, but will induce an undesired additional flow of ambient air into the device. The latter would lead to an unwanted (greater) dilution of the breath sample, which must be avoided as it would further decrease the methane concentration of the sample. Increasing the airflow rate must therefore be done with care.

Nonetheless, increasing the airflow rate in parallel to finding the optimal inlet surface area and resistance levels in the openings could have a simultaneous, positive effect by increasing the relative negative pressure effect under the hood. A relative under-pressure induces an inflow from the higher pressure environment to the under-pressurized one, ensuring a greater breath recovery. The relative under-pressure we wished to achieved in Chapter 5 may not have been reached in the current setting. It could be increased by augmenting the airflow rate and decreasing the surface area of the inlets and the resistance in the openings (*e.g.* using soft materials, avoiding sharp edges). As the negative pressure patterns obtained in the previous design of the CHS were shown not to be homogeneous in space (even at the same height plane) (Wu, 2016), its dynamics should be evaluated.

Additional factor affecting recovery: head position

It is now well established that the head position of an animal within an unclosed gas exchange monitoring system has a significant impact on breath recovery, and thus on the accuracy of the resulting MPR estimate. This does not represent an issue for closed monitoring systems (*e.g.* CRC, PAC), as they use total air sampling (Hegarty, 2013). However, it is a problem when gas concentrations are measured in air samples of unclosed monitoring systems (*e.g.* GreenFeed emission monitoring (**GEM**), CHS, sniffers), with aerial CH_4 and CO_2 concentrations being influenced by the distance between the sampling inlet and the head (mouth and nostrils) of the animal (Lassen et al., 2012). Huhtanen et al. (2015a) experimented with the effect of muzzle angle and distance to the inlet of a GEM and a sniffer. They concluded that even small alterations of the head position of the (simulated) animal induced large differences in measured gas concentrations, with a greater effect on the concentration (*i.e.* sniffer) than the flux (*i.e.* GEM) method. The same phenomenon was observed in the CHS.

In Chapter 5, the choice was made not to filter observations based on head position to maximize sample size. However, using this dataset, the head positions of cows were assessed by an observer based on the video recordings of the experiment (not reported in this thesis). A selection of observations was made according to the fraction of time during which the head of the cow was positioned forward, near the extraction inlets under the hood (head-body angle between 90 and 180°). After filtering, only the data of the cows that still had more than 30 measurement cycles (**MC**) were kept (n = 7 cows). The mean MPR estimated over these MC was compared to the ones made by the CRC for the same cows. A linear regression yielded a Pearson's *r* correlation of 0.401, while *r* equated 0.257 when observations were not filtered based on head position. While this does not drastically improve the correlation between CRC and CHS estimates, it does show clearly that muzzle position has an effect on the MPR monitored by the CHS. This also allowed to quantify the level of bias present. The mean methane concentrations of all (21 sec) measurements (Figure 6.2) taken with cows in frontwards positions (head-body angle between 90 and 180°, n = 2528 observations) equaled 130.9 ± 8.8 (sd) ppm, while they equated 65.2 ± 12.2 ppm in backwards positions (0-90°, n = 430). This represents a mean difference of 65.7 ppm between both positions. Being in line with the literature, these results emphasize the importance of monitoring the head position (and filtering observations according to it) as a step towards obtaining more accurate estimations of MPR with unenclosed devices.

Making use of this information is not only important for unenclosed devices in terms of measurement accuracy, but also because ignoring the effect of head pose on enteric methane measurements might also lead to the (genetic) selection of presumably low producing animals, which are in fact simply more restless or position their head farther away from the inlet of the monitoring device (Hammond et al., 2016a).

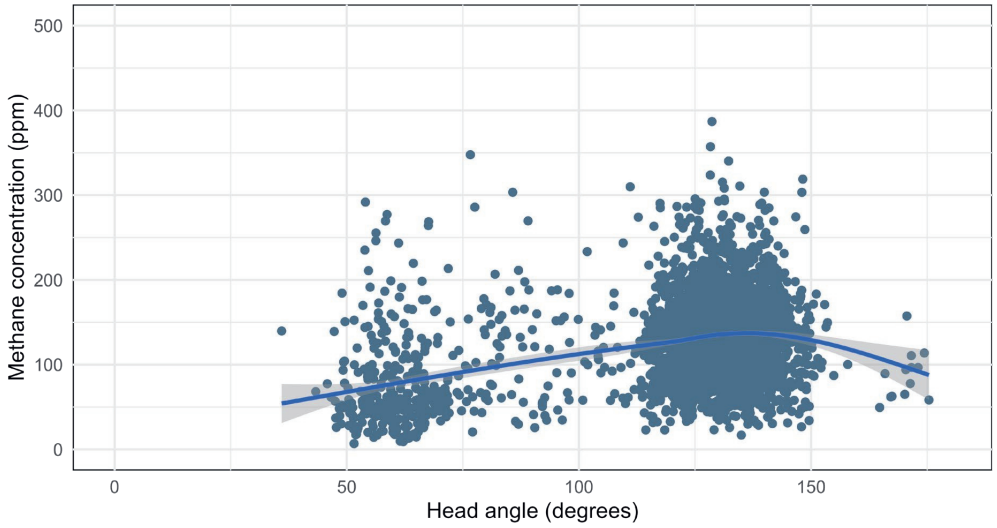


Figure 6.2. Breath methane concentrations measured by the cubicle hood sampler depending on the angle of the head under the system.

6.3.2. Background concentration measurements

When measuring methane emissions or fluxes, background concentrations (from the herd or the manure pit) have to be monitored so that they can be subtracted from the content of the breath of the animal(s) of interest. Errors in background concentration measurement will result in inaccuracies in MPR estimates [eq. 4.2]. These errors can be linked to three main factors.

Sampling location and sample representativeness

Errors can be made if the positioning of the background sampling points does not allow for a representative monitoring of the methane concentrations near the inlet of the monitoring device. For example, in their study using SF_6 as a tracer gas, Berndt et al. (2020) showed that most (but not all) cows tended to group in the pasture, and that the monitoring of CH_4 and SF_6 concentrations within this pack led to significantly higher background concentration levels than the ones monitored farther away in the paddock. Therefore, subtracting background concentrations measured farther away from the individual production of the animals that tend to pack up could lead to an overestimation of the individual MPR. Conversely, deducting background levels measured in the pack from the production of isolated animals could lead to an underestimation of their MPR.

A similar phenomenon might have been observed in Chapter 5. The decision was made to monitor background methane concentrations at the rear end of the cubicles (Figure 6.5 red dots). This location, a compromise between sufficient distance from the source and representativeness of the background air flowing into the hood, should allow to detect and monitor the methane concentrations patterns close to the hood. However, these sampling points were positioned near the slatted floor and manure pit. There is thus a chance that methane emitted by manure led to inhomogeneous spatial concentrations patterns, and was sensed by the background sampling points, leading to an overestimation of the methane concentrations in the background air entering the hood. A possibility to prevent this could be to place the sampling points higher and closer to the front of the cubicle, while maintaining them out of cows reach to avoid damage. In any case, finding the optimal location to monitor background concentrations is a challenge, and flow dynamics (spatial and temporal variation) occurring around and within the CHS should be extensively investigated.

Another factor that could affect the representativeness of the sample is the sampling scheme. Wu et al. (2016) showed the presence of great temporal and spatial variations in methane concentrations around a preceding prototype of the CHS. They also added that the coefficient of variation (**CV**) observed between background measurements strongly increased the CV of the hood's measurements. It might therefore be preferable to monitor both hood and background concentrations simultaneously to better capture the temporal changes occurring in the background. The impact of simultaneous versus non-simultaneous background sampling remains to be investigated, and might require the availability of multiple gas analysers or sensors.

Interference of surrounding cows

During the experiment conducted in Chapters 4 and 5, it appeared that cows surrounding the cubicle being monitored could impact the background methane concentrations measured. For example, animals tend to stand or walk by the CHS, sometimes with their muzzles positioned near the background sampling points (Figure 6.3). Other problematic situations arose from the position of cows lying in cubicles that were adjacent to the one being monitored. When rolled up (Figure 6.4), animals were exhaling directly under the background sampling points, potentially leading to the measurement of higher background concentrations which most likely did not represent the true ambient levels. These interferences correspond to random errors that

could be ignored as they do not systematically occur. However, if they prove to impact the MPR estimations too greatly and too regularly, it would be interesting to filter them out.

An approach to this issue would be to relocate the background sampling points. Although not reported in this thesis, multiple background sampling points were tested in an experiment conducted in the absence of cows. For this test, ten background sampling points were installed at 10 different locations (Figure 6.5), and their ability to detect various (mimicked) interferences of cows was evaluated. It turned out that none of them was able of sensing all the events simulated, but that a positioning alternative to the one used in Chapters 4 and 5 was the one that detected the greatest number of them. The positioning of this sampling point is visible in Figure 6.5 (blue dot). This experiment showed that the background sampling points used in the research carried out (red dots, Figure 6.5) were perhaps not the most appropriate ones to sense cow interference, and that this could have led to errors in the monitoring of the background and (consequently) in the MPR estimated by the CHS. Nonetheless, this conclusion may not be extendable to the adequacy of the background sampling points when monitoring concentrations without surrounding cows as it was not investigated.

Figure 6.3. Example of a surrounding cow standing and exhaling onto the background sampling point (green circle) of the monitored cubicle hood sampler.

Figure 6.4. Example of a surrounding cow (left) lying in a rolled-up posture and exhaling underneath the background sampling points (red circles) of the monitored cubicle hood sampler.

Figure 6.5. Background sampling points tested when evaluating the effect of surrounding cows on the monitored background concentrations. Blue dot: most suited background sampling point. Red dots: current sampling points. Green dots: other background sampling points tested. Ambient sampling point: not visible on this picture. Credit: W. Westerbeke.

Hood-background concentrations difference

Aiming for the largest hood-background concentrations difference is a way to reduce the effect of uncertainties in background measurements on the MPR estimate. This is because highly concentrated breath will be less sensitive to errors in background concentrations if they represent a relatively small percentage of the sampled breath concentration. This was shown by McGinn et al. (2021) with GEM, whose high uncertainty in background concentrations had little influence on the simulated breath measurements thanks to the large difference between the two mean concentrations. In our experiment with the CHS, the methane concentrations measured at the background points equated 35.3 ± 26.9 (sd) ppm in average, while the breath samples captured by the hood had a mean concentration of 125.6 ± 56.0 ppm. Even without accounting for the spread of values, this means that (on average) the measured background levels are equal to 28.1% of the mean breath concentrations. In this setup, it means that variation or uncertainty in background measurements will considerably affect the resulting MPR estimate. Rethinking the settings of the design to increase concentration differential therefore seems necessary.

6.3.3. Whole-system recovery test

Gardiner at al. (2015), followed by Hammond et al. (2016a) and Gerrits et al. (2017), have stressed the importance of conducting recovery tests prior to any gaseous exchange measurements and of reporting their results. As they declared, it has become apparent that not all research groups adhere to the same standards, and that even the gold standard CRC can yield significantly different recoveries rates between facilities if not regularly checked (Gardiner et al., 2015). The results of Gardiner et al (2015) showed that, in the case of CRC, most of the absolute uncertainty was due to the facility design and the operation mode. Instrumental noise or chamber-to-chamber variability appeared to account for a smaller portion of the uncertainty (Gardiner et al., 2015). Most of the variation proved to be actually due to uncertainties in the sample ducting and flow measurements (15.3%), the chamber mixing (3.4%), and in the methane analyser measurements (1.3%). These results show that full-system recovery tests must be conducted (Gardiner et al., 2015) and not sole calibrations of parts of the system, a need that was also stressed by McGinn et al. (2021).

Conducting this type of recovery tests can be fairly easily implemented in measurement procedures as they are relatively simple to perform. This was shown in Chapter 2 (PAC) and 4

(CHS). They can allow to detect and quantify the presence of systematic and random errors, which characterize the deviation of the result from the true value (Rabinovich, 2006). When they classify as random errors, they can increase the variance of the individual measurements (called noise) without altering the mean. In this case, they directly translate into an uncertainty, but the effect of these errors can be reduced with larger sample size (Aubinet, 2023). Systematic errors, on the contrary, do not generate noise but induce a bias in the measurements. Corrections can be applied if the source of error can be identified and quantified (Aubinet, 2023). Unfortunately, it is not always possible to do so and, in the worst case, unidentified systematic errors may remain. This kind of systematic error probably occurred in the comparative study of Chapter 5, but was not detected in the recovery tests described in Chapter 4. This shows that although recovery tests provide valuable information on the performance of a system itself and should be conducted (Chapter 2, Chapter 4), they may not be fully representative of the actual experimental conditions, nor be indicative of the system's ability to recover breath samples in the presence of animals.

6.3.4. Sampling scheme and data analysis

Sampling scheme

Postprandial MPR has been demonstrated to be generally non-linear and inherent to time (Crompton et al., 2011; van Lingen et al., 2017), with eaten quantities, ingestion speeds and feeding frequencies affecting the amplitude and length of each phase of the methane production curve (minimum, increase, maximum, decrease). These factors have been shown to play a crucial role in the number of daily samplings and the intervals that are necessary for spot sampling techniques to obtain reliable methane production estimates (Lee et al., 2022; van Lingen et al., 2023). Indeed, Lee et al. (2022) used CRC data to show that at least eight samples (every 3-h over a 24-h period) were necessary to estimate daily CH_4 production, and to detect changes in MPR linked to dietary treatments. Van Lingen et al. (2023), for their part, stressed the need of defining accurate sampling schemes in connection to the feeding regime when estimating daily enteric methane production from cattle. They reported that at least three equally spread measurements should be taken each day when animals are fed ad libitum twice daily. When feeding was restricted (cows fed twice daily at 80–95% of the ad libitum intake), measurements taken at least every hour were necessary to obtain accurate MPR. This can be linked to the fact that the hourly MPR decreases as the number of feed deliveries in a day increases (Crompton et al., 2011). Defining a spot sampling scheme for monitoring enteric

methane must therefore be done with respect to the feeding regime that is sustained. Besides, it is important to note that the monitoring device used by Lee et al. (2022) and Van Lingen et al. (2023) to reach these conclusions was the CRC. It is possible that the method used to assess the measurement accuracy in connection to the sampling scheme may have an effect. Other conclusions in terms of sampling frequency might therefore be reached for each monitoring devices. Most likely, we can presume that devices operating at accuracy levels that are lower than the CRC will require more frequent sampling than the gold standard.

Moreover, some studies have reported MPR to be the lowest before the ingestion of the first meal of the day (Hammond et al., 2016b; Laubach et al., 2013). This points to the necessity to balance measurements over days and nights (van Lingen et al., 2023). This could be due to differences in feeding and ruminating behaviours observed between day time and night time (Schirmann et al., 2012). Daily MPR has also been demonstrated to vary between days, with Grainger et al. (2007) finding a variability (CV) of 4.3% within cows (n = 28) amongst days (n = 3), and Blaxter and Clapperton (1965) reporting a 7.2% CV for day-to-day (n = 4 to 5) variation based on 24-h monitoring of MPR in cattle (n = 54). This raises the question of the minimal period of time (days) over which an animal should be monitored for its MPR estimate to be representative of its true production, especially when linked to varying metabolic rate (*e.g.* heat load, activity, time of the day) and metabolic state (*e.g.* pregnant, lactating).

Data analysis

Statistical analysis and modelling are powerful tools that can prove useful when estimating daily MPR based on spot measurements. The model proposed in Chapter 3 provides an interesting approach to converting spot measurements into continuous methane production curves. Although it could not compensate for the biased estimates made by the CHS in Chapter 5, it might improve the MPR estimation accuracy when fitted on unbiased data. By borrowing information across individuals of a same population, it could allow monitoring devices to deal with less frequent observations. In addition, this model accounts for the variability in MPR that is known to be present between cows, making its estimates realistic. Lastly, it allows to approach daily MPR as the estimated area under the curve (**AUC**), which better represents the dynamics of the production process than a mean does. Similarly, the use of smoothing splines was proposed by van Lingen et al. (2023) to fit the observations made in CRC before computing an AUC. Interpolation might also be an approach to compensate for the gaps present between two measurements, for example when monitoring background concentrations. However,

statistical tools should be used cautiously, and the excessive use of adjustments and correction factors should be avoided in the case of enteric methane assessments.

6.3.5. Overall interconnectedness of the sources of uncertainty

In addition to identifying the elements responsible for most of the uncertainty that is linked to the practical monitoring of individual methane production, it is essential to stress their interconnectedness. A poor recovery of the breath sample by the monitoring device will, in some methods, simultaneously lead to an overestimation of the background concentrations. Similarly, the presence of cows around the system can affect both the ongoing background measurement, for example by exhaling air near its inlets, and lead to the overestimation of the methane concentrations contained in background air. Both cases would lead to an underestimation of the methane production of the monitored animal. A sampling scheme that does not allow to capture the different phases of the postprandial methane production curve would lead to biased estimates of the individual MPR. Data processing can be helpful to partially compensate for missing information, but it cannot compensate nor correct for everything. The statistical analysis carried out should be also be defined depending on the nature of the dataset. Additionally, omitting to conduct whole-system calibrations will prevent the results from being corrected for systematic errors. Conversely, even the most accurate monitoring device cannot provide accurate estimations of MPR if the breath sample it analyses is not representative of the animal's production or if the sampling scheme is inadequate. The overall connections involved are shown in Figure 6.6.

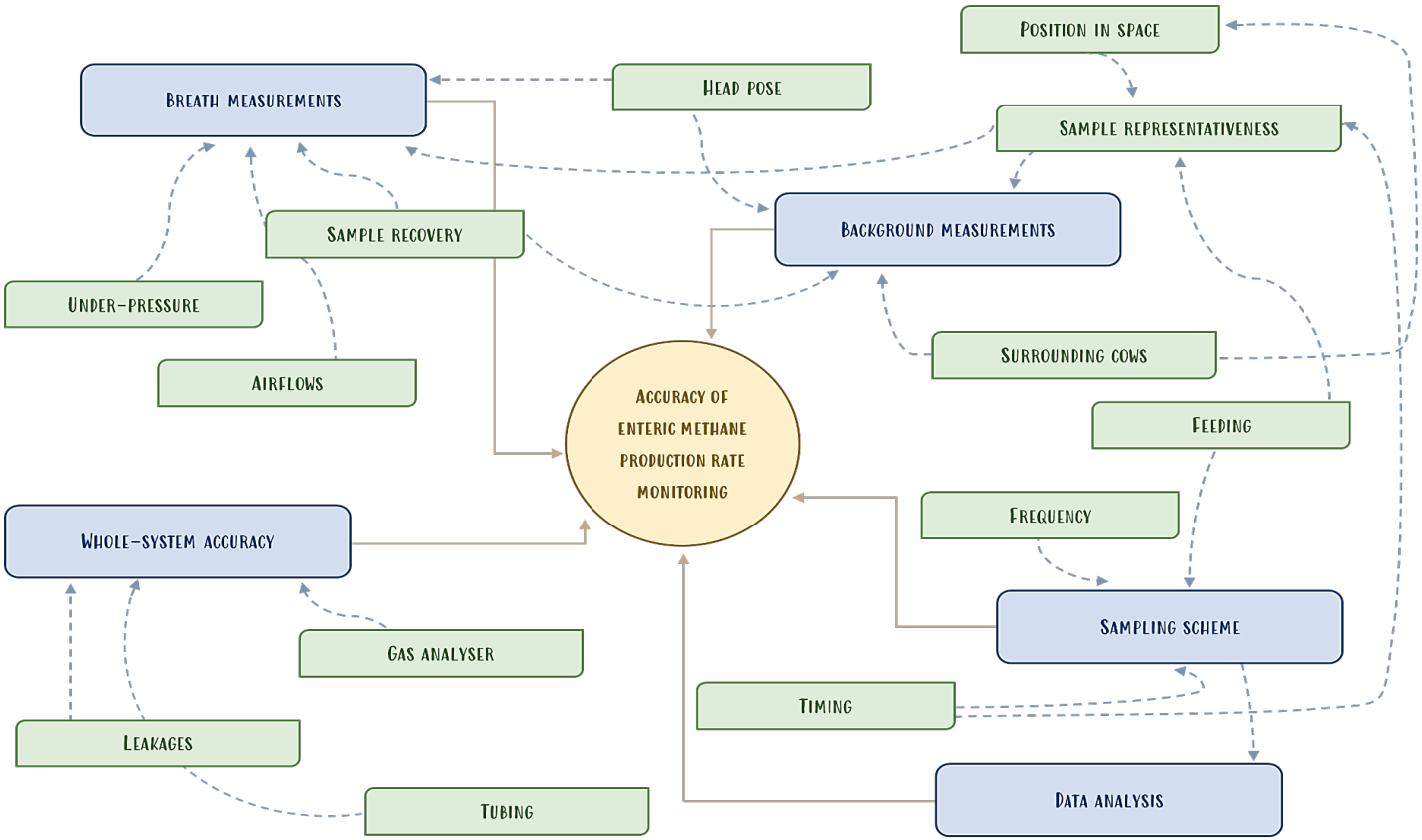


Figure 6.6. Schematic overview of the systemic challenges related to the practical monitoring of enteric methane production. Plain arrows represent the effects of the key factors (blue boxes) on the accuracy of the enteric methane production rate monitored (yellow circle). Dashed arrows indicate the effect of sub-factor (green boxes) on key factors (blue boxes).

6.4. Remaining unknows and further outlook

This study has brought to light the many challenges of monitoring enteric methane production of ruminants under practical conditions. On the basis of these elements, proposals are made for further research in order to try to solve some of the issues that were encountered with the CHS and to obtain a better understanding of the underlying processes.

Breath recovery

As extensively discussed, the breath recovery capacity of the CHS must be investigated. Experiments should be conducted to assess airflows patterns within the system and link them to the potentially poor under-pressure effect induced under the hood. A possibility could be to evaluate recovery at (varying) higher airflow rates and smaller inlet surfaces than the ones previously tested. This should be done in a recovery test, but also in the presence of animals. For the latter, animals could be equipped with tubes placed close to their muzzle and releasing a tracer gas (*e.g.* SF_6) to evaluate the portion of this gas that is recovered by the hood.

Background sampling

Errors made in background sampling must be evaluated and addressed. First, the current sampling points proved not to be the best suited to detect the interference of surrounding cows. There is a chance that they were also not the most optimal to monitor the methane concentration patterns occurring around the CHS (varying in space), notably in connection with the potential interference of the manure pit emissions. These sampling points could eventually be relocated. However, relocation must be done with care as background measurements and dynamics have been shown to be complex. Firstly, airflows dynamics within and around the cubicles could be investigated with smoke tests. This could allow to visualize general flow dynamics and to (ideally) detect the presence of problematic patterns (*e.g.* emissions from the manure pit flowing onto the background sampling points). Secondly, using a tracer gas to assess the fraction of gas recovered by the hood (detailed in the *Breath recovery* outlook) would also allow to evaluate the fraction of the breath that is drawn back to the background samplings points. In addition, the relative effect of background measurements uncertainties could be decreased by increasing the differential between hood and background concentrations. This could be achieved by recirculating the air captured by the hood, as done in the CRC. Additionally, background air might require to be mechanically mixed, so that the effect of

sporadic events (*e.g.* surrounding cows), temporal and spatial variations can be smoothed out. Lastly, the effect of alternative and simultaneous monitoring of background and breath concentrations should be assessed. This would allow to evaluate its effect on the accuracy of the resulting MPR estimate.

Sampling scheme

Studies conducted in CRC have investigated and showed the importance of the sampling scheme (within and between days) when doing spot sampling measurements. This has not yet been investigated for other monitoring devices. Before investigating the effect of sampling scheme on the MPR estimated by practical devices, whole-system calibrations should be conducted to correct for bias. Then, multiple sampling frequencies could be experimented with and compared to the MPR levels monitored by CRC for the same animals. This will prove challenging as the levers of actions to increase the throughput of practical devices are limited. In a first time, it might be necessary to enclose animals so that their MPR is monitored over at least an entire 24-h period. In the case of the CHS, it could be achieved by building a CHS into tie-stalls to monitor a few individual cows over multiple days. Using these datasets, increasing percentages of records could be deleted to evaluate the effect on the resulting MPR estimates. If it appears that more observations are needed than what the positioning in the barn allows (*e.g.* cows not lying down right after eating and at certain moments of the day), we might consider redesigning the system. An inevitable additional layer of complexity will be added with varying feeding regimes. Its general effect should be investigated similarly to the studies of Lee et al. and van Lingen et al. (2022; van 2023). Trends might be obtained, but it will not be possible to evaluate the effect of all regimes (*e.g.* content, quantities) and feed intake patterns (*e.g.* speed, frequency).

Hierarchical methane rate model

Fitting the HMR model with CHS data did not lead to a significant improvement of the accuracy of the MPR estimates due to the presence of a strong measurement bias. However, HMR proved its usefulness on a CRC dataset. It would therefore be informative to investigate its added value when used with unbiased CHS records, and with the observations of other spot sample methods. It would be particularly ideal if the model could allow to (at least partially) compensate for the small sample size collected by some of the practical devices. Investigating minimal input sample size could be evaluated by fitting the HMR model with CRC data, and

comparing its output with the mean values monitored (as in Chapter 3) after randomly deleting increasing numbers of observations. While the number of observations most certainly plays a role on the estimation accuracy of HMR, it might also be affected by the phase of the postprandial methane production curve during which they were collected. For example, it could be that only the first 120 minutes after feed intake are of interest as they correspond to the minimum and the maximum production levels, with this duration depending on feeding regime (*e.g.* frequency, speed, quantities).

In the present study (Chapter 3), feeding regimes were known and consistent over cows. Different feeding frequencies would alter the shape of the postprandial production curve, which would alter the parameter values of the HMR model. The dependency between estimation accuracy and varying feeding regimes should be explored.

Additionally, it would be informative to reconduct the model validation of Chapter 3 after allowing all parameters (shape and scale) to vary between cows. While being computationally more expensive than the partially pooled model, it could allow to identify which parameters vary between individuals and to what extent. This might enhance the accuracy of the MPR estimated by HMR. Finally, days could be included as an additional level of hierarchy to the model so that the between-days variation in MPR (*e.g.* within cows) can be taken into account.

Head position monitoring

If higher recoveries of breath are achieved in the future the head pose of animals under the hood would become less of an issue. As this is not yet the case and because completely rolled up animals would most likely remain an issue, monitoring head pose remains of importance. Through the experiments, many sensors aiming at monitoring head position have been tested (*i.e.* infrared, ULS, RFID). Unfortunately, none of them (in the tested setup) yielded satisfying performances. Recently, a pose estimation algorithm was trained to detect key points of cows (*i.e.* muzzle, ears, head, shoulders, cross bone) and calculate the angle between them (Figure 6.7, Figure 6.8). This information could be used after data collection to filter out observations recorded in inadequate postures. These results will be communicated at a later stage, but the first results appear very promising.

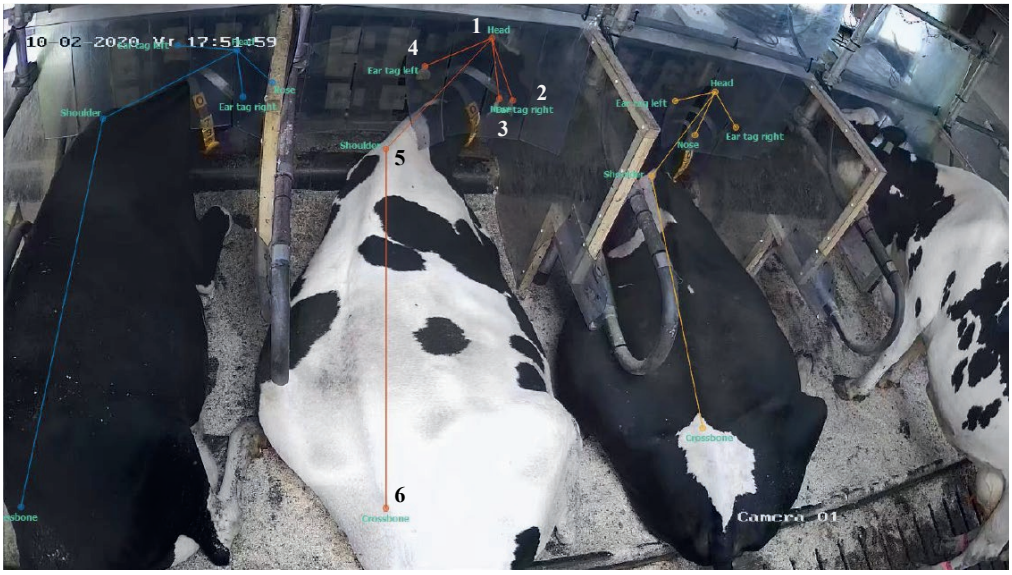


Figure 6.7. Simultaneous key points detection of the head (1), muzzle (2), right (3) and left (4) ears, shoulder (5) and crossbone (6) of three cows lying in cubicle hood samplers. Credit: J. Blom.

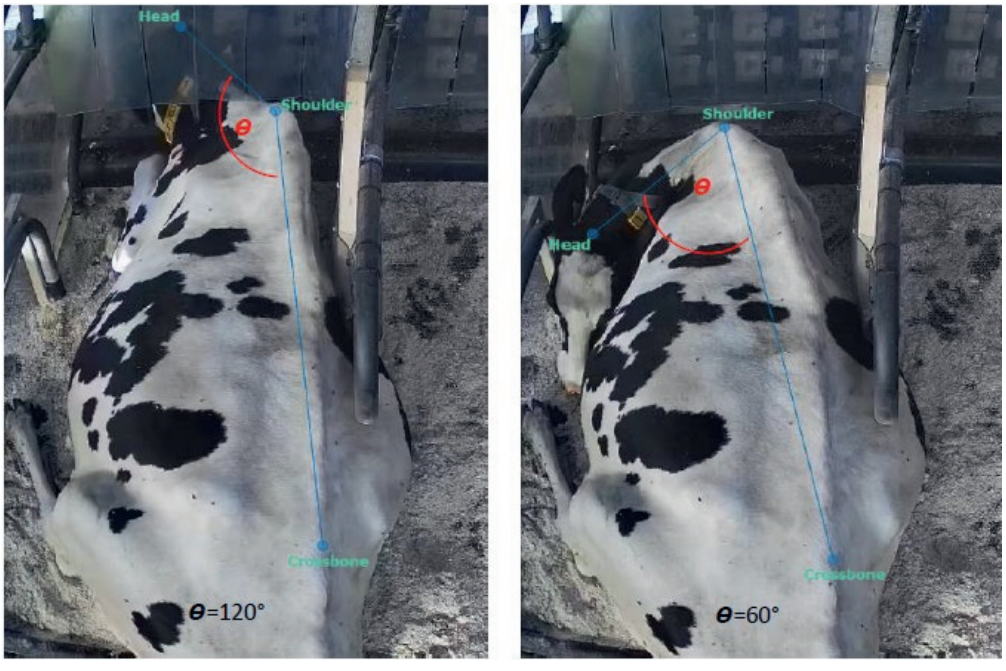


Figure 6.8. Examples of two head angles (left θ = 120 degrees, right θ = 60 degrees) calculated by the algorithm for the same cow while lying a cubicle hood sampler. Credit: J. Blom.

Cow identification

Attributing observations to the correct cow is essential to avoid errors made in estimating individual MPR. In the tested setups, RFID did not yield a sufficient performance (*i.e.* identification accuracy and robustness). However, technical advances are being made, and more robust radio frequency identifiers are now available on the market. While they could be an option to consider, an alternative is to opt for a computer vision algorithm which would limit the use of sensors in the barn (security cameras being cheaper and less sensitive to barn environments than the RFID sensors). Reusing the open source work of Andrew et al. (2020, 2016), cows might be identified using their coat patterns (Figure 6.9). Challenges will most likely emerge when identifying the mostly unicolour cows, and from the varying angles at which cows can be seen depending on their lying position (and flank). An alternative idea could be to identify the number on the ear tags of the animals as they enter a CHS.

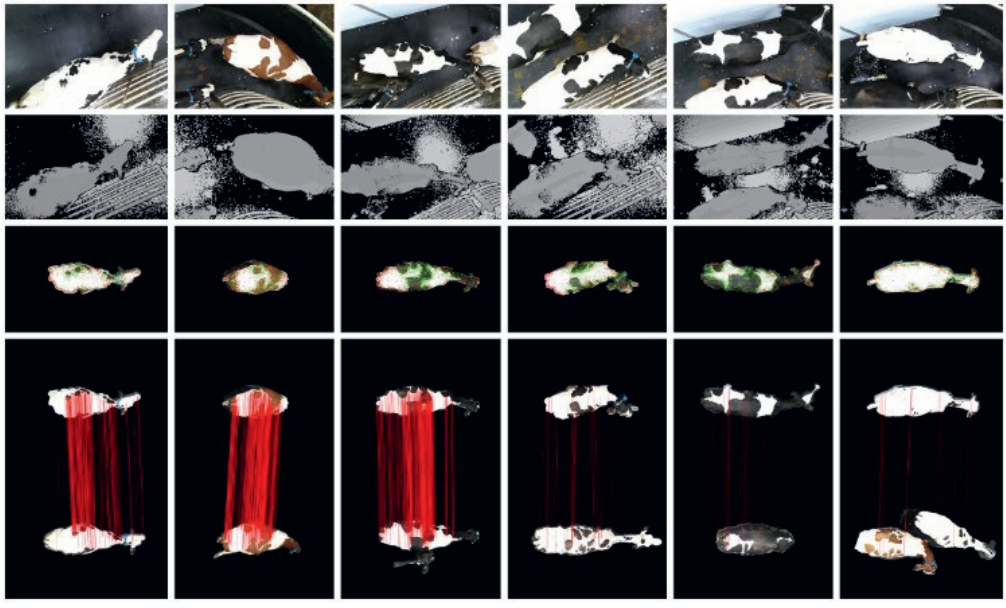


Figure 6.9. Examples of identification process with successful (first three columns) and unsuccessful (last three columns) image-pair comparisons (Andrew et al., 2016). Row 1: RGB images and row 2: corresponding depths images. Row 3: images yielded from pre-processing with retained and discarded features following feature-importance prediction in green and red respectively. Rows 4 and 5: examples of feature matching and geometric filtering on the same individual (left 3 examples) and different individuals (right 3 examples).

6.5. Concluding remarks:

Monitoring individual enteric methane production rates under practical conditions represents a challenge in compromising required measurement accuracy with practical farm conditions. Many difficulties emerge from the barn environment (*e.g.* dust, humidity, non-linear airflows, other methane sources) and the presence of animals (*e.g.* voluntary movement, physiological processes, damage). Management strategy, farm type and design, ruminant species, and feeding regime are few of the many factors that vary greatly and fundamentally affect what can be done to monitor MPR in practice. It is therefore unlikely that there will ever be a "one size fits all" device to monitor enteric methane production rates of ruminants in practice.

Conversely, respiration chambers were shown to be ideal for gas exchanges measurements. They provide the possibility to control many factors so that only the variables of interest can be explored. Unfortunately, they do not permit the large screening of animals, which remains a necessity. There is currently no consensus on one practical device that operates at sufficient levels of accuracy and throughput, but the need for large-scale screening does not justify the use of inaccurate or biased devices. Efforts to monitor enteric methane production rates in practice should therefore continue. Anyone striving to achieve more accurate measurements of the individual enteric methane production of animals in practice should therefore make conscious decisions concerning the following key elements:

- Breath sample recovery and representativeness;
  - *Taking into account that if the exhaled breath of the animals is not sufficiently recovered by the monitoring device, the sample being analysed will not be representative of the true methane contained in the breath, thus leading to biased methane production estimates.*
- Background measurements representativeness;
  - *To avoid misestimating background concentrations, for example by not monitoring the true background concentrations of the air present near the monitoring device, which would lead to an under or over-estimation of methane production.*
- Whole-system performance and accuracy;
  - *As the estimates that are made by a monitoring device that has not been calibrated cannot be corrected for measurement errors and can lead to bias.*
- Overall sampling scheme and data handling;

- *Which should be defined so that the general non-linearity of the methane production curve can be captured by the device and appropriately taken into account in the data analysis.*

Lastly, it is strongly recommended to address these key elements in a systemic manner as their interconnectedness has been demonstrated in this thesis (Figure 6.6).

Supplementary material

Appendix A. Detailed ring test PAC.

Table A.1. Ring test: Monitored methane concentrations (ppm) post-injection, per chamber and per repetition. Mass injected during the ring test (1.6 g) were injected in triplicates in each of the eight chambers. One replicate (PAC 6 replicate 1) had to be removed from the analysis as the fan was not activated during the measurement, but is present in this table (indicated as "OTL"). Supplementary information relative to methane volume (L) and mass (g) injected calculated from [eq. 2.2] to [eq. 2.4] are also presented. Recovery rates (%) were calculated from [eq. 2.5].

PAC number	Replicate	Injected mass (g)	Monitored [CH_4] post-injection (ppm) Mean ± sd	Corresponding CH_4 volume injected (L)	Estimated CH_4 mass recovered (g)	Recovery rate (%) ± sd between measurements
2	1	1.56	1400.00 ± 0.00	1.58	1.08	69.33 ± 0.00
	2	1.56	1400.00 ± 0.00	1.58	1.09	70.18 ± 0.00
	3	1.56	1400.00 ± 0.00	1.58	1.08	69.38 ± 0.00
3	1	1.58	1400.00 ± 0.00	1.58	1.08	68.63 ± 0.00
	2	1.57	1400.00 ± 0.00	1.58	1.09	69.61 ± 0.00
	3	1.61	1450.00 ± 0.00	1.63	1.12	69.49 ± 0.00
4	1	1.62	1450.00 ± 0.00	1.63	1.13	69.59 ± 0.00
	2	1.57	1350.00 ± 0.00	1.52	1.04	66.58 ± 0.00
	3	1.57	1400.00 ± 0.00	1.58	1.08	69.09 ± 0.00
5	1	1.58	1450.00 ± 0.00	1.63	1.13	71.65 ± 0.00
	2	1.58	1400.00 ± 0.00	1.58	1.08	68.63 ± 0.00
	3	1.59	1400.00 ± 0.00	1.58	1.08	68.13 ± 0.00
6	1: OTL	1.58	1183.33 ± 23.57	1.33	0.92	57.97 ± 1.15
	2	1.63	1400.00 ± 0.00	1.58	1.10	67.55 ± 0.00
	3	1.59	1400.00 ± 0.00	1.58	1.08	68.13 ± 0.00
7	1	1.56	1400.00 ± 0.00	1.58	1.08	69.57 ± 0.00
	2	1.56	1400.00 ± 0.00	1.58	1.10	70.55 ± 0.00
	3	1.56	1400.00 ± 0.00	1.58	1.08	69.38 ± 0.00
8	1	1.59	1433.33 ± 23.57	1.62	1.12	70.39 ± 1.16
	2	1.59	1433.33 ± 23.57	1.62	1.11	69.75 ± 1.14
	3	1.63	1500.00 ± 0.00	1.69	1.16	71.17 ± 0.00
10	1	1.56	1400.00 ± 0.00	1.58	1.09	70.01 ± 0.00
	2	1.56	1400.00 ± 0.00	1.58	1.08	69.38 ± 0.00
	3	1.56	1400.00 ± 0.00	1.58	1.08	69.38 ± 0.00

Appendix B. Detailed range test PAC.

Table A.2. Range test: Monitored methane concentrations (ppm) post-injection, per chamber and per repetition. Mass injected during the range test (0.5, 1.0, 2.0 and 2.5 g) were injected in duplicates in each of the two chambers. Subset of the ring test (1.5g) is added to the range test data for better readability. Ring tests injections were done in triplicates. One replicate (PAC 6 replicate 1) had to be removed from the analysis as the fan was not activated during the measurement, but is present in this table (indicated as "OTL"). Supplementary information relative to methane volume (L) and mass (g) injected calculated from [eq. 2.2] to [eq. 2.4] are also presented. Recovery rates (%) were calculated from [eq. 2.5].

PAC number	Injected mass (g)	Replicate	Monitored [CH_4] post-injection (ppm) Mean ± sd	Corresponding CH_4 volume injected (L)	Estimated CH_4 mass recovered (g)	Recovery rate (%) ± sd between measurements
5	0.47	1	463.33 ± 4.71	0.52	0.36	74.52 ± 0.76
		2	460.00 ± 0.00	0.52	0.35	74.13 ± 0.00
	1.06	1	1000.00 ± 0.00	1.13	0.77	71.48 ± 0.00
		2	1000.00 ± 0.00	1.13	0.77	71.48 ± 0.00
	1.53	1	1450.00 ± 0.00	1.63	1.13	71.65 ± 0.00
		2	1400.00 ± 0.00	1.58	1.08	68.63 ± 0.00
		3	1400.00 ± 0.00	1.58	1.08	68.13 ± 0.00
	2.01	1	1850.00 ± 0.00	2.08	1.43	69.98 ± 0.00
		2	1850.00 ± 0.00	2.08	1.43	69.98 ± 0.00
	2.48	1	2266.67 ± 23.57	2.55	1.75	69.39 ± 0.72
		2	2266.67 ± 23.57	2.55	1.75	69.53 ± 0.72
6	0.47	1	450.00 ± 0.00	0.51	0.35	72.37 ± 0.00
		2	463.33 ± 4.71	0.52	0.36	74.52 ± 0.76
	1.06	1	1000.00 ± 0.00	1.13	0.77	71.48 ± 0.00
		2	1000.00 ± 0.00	1.13	0.77	71.48 ± 0.00
	1.56	1: OTL	1183.33 ± 23.57	1.33	0.92	57.98 ± 1.15
		2	1400.00 ± 0.00	1.58	1.10	67.55 ± 0.00
		3	1400.00 ± 0.00	1.58	1.08	68.13 ± 0.00
	2.01	1	1850.00 ± 0.00	2.08	1.43	69.96 ± 0.00
		2	1850.00 ± 0.00	2.08	1.43	70.10 ± 0.00
	2.48	1	2250.00 ± 0.00	2.54	1.73	68.83 ± 0.00
		2	2250.00 ± 0.00	2.54	1.73	68.97 ± 0.00

Appendix C. A primer on Bayesian data analysis.

The Bayesian viewpoint provides an alternative to the classical approach to data analysis. Let θ denote a model-specific collection of parameters (of continuous metric). The classic approach to data analysis then uses inferential procedures based on the likelihood of the observed data $L(\theta; X)$. This likelihood represents the joint distribution of the data X under the parameters of the postulated model. Usually, a retrospective evaluation is made of the estimate $\hat{\theta}$ over all possible X values conditional on the true unknown θ which is deemed fixed. Hence, in this viewpoint, the parameter collection is fixed and the data are random. The Bayesian approach, however, considers θ random. This allows one to construct $\pi(\theta|X)$, the posterior distribution of the model parameters given the observed data. One then has to specify the prior distribution $\pi(\theta)$, reflecting the uncertainty or knowledge regarding the model parameters before being witness to the data. One can then use Bayes' rule to obtain the posterior distribution of interest:

$$\pi(\theta|X) = \frac{L(\theta; X)\pi(\theta)}{\int L(\theta; X)\pi(\theta)\, \partial\theta}.$$

The posterior distribution thus consists of the joint probability function for θ and X (the numerator in the expression) weighted by the marginal probability of the data under the model (the denominator, also called the prior predictive density or marginal likelihood). Intuitively, the Bayesian posterior is a weighted calibration of our data evidence with our prior knowledge.

Bayes' rule encapsulates the core machinery of Bayesian statistics. The denominator, however, often cannot be solved analytically, meaning that the posterior is not available in closed form. Posterior evaluation is then possible with the use of Markov chain Monte Carlo (MCMC) methods. MCMC represents a class of algorithms generating sequences of random samples that converge in distribution to a target probability distribution from which one cannot sample directly. In this work we use the powerful Hamiltonian Monte Carlo approach.

The Bayesian approach to data analysis has several distinct advantages, several of which we will state here. First, parameter uncertainties are propagated (using MCMC) throughout the model, ultimately making for more robust and realistic model performance and model predictions. Second, it allows one to combine expert knowledge (in the form of prior formulations) with data evidence. One can thus incorporate multiple sources of information. Third, it has extensive model comparison capabilities allowing for a better grasp on the important question how appropriate a model is given the collected data. Fourth, it allows for very flexible (hierarchical) model construction. This work, for example, shows that it is very

permitting in implementing a physical model in a hierarchical stochastic setting. For more information on the Bayesian approach to data analysis we confine by referring to Gelman et al. (1995), Lambert (2018), and Press (2002).

Appendix D. Detailed recovery tests CHS.

Table A.3. Summary table of the injected and recovered methane mass for each of the recovery test conducted. Treatment 1 represents a target daily MPR of 200 g/d, and treatment 2 of 400 g/d. Corresponding ambient conditions are also stipulated. Airflow corresponds to the mean flow going through the hood. Temperature was the ambient value in the barn.

CHS	Repetition	Treatment	Injected CH_4 mass (g)	Recovered CH_4 mass (g)	Recovery rate (%)	Mean airflow (m^3/h)	Temperature (°C)
1	1	1	192.8	228.0	118.2	197.1	20.1
1	1	2	385.6	392.6	101.8	197.1	20.0
2	1	1	192.8	235.9	122.3	196.9	20.2
2	1	2	385.6	439.9	114.1	196.9	20.3
3	1	1	192.8	204.9	106.3	186.1	20.5
3	1	2	385.6	386.8	100.3	186.1	20.5
4	1	1	192.8	227.6	118.1	180.0	20.3
4	1	2	385.6	415.2	107.7	180.0	20.4
1	2	1	194.8	224.0	115.0	197.7	12.8
1	2	2	389.5	405.4	104.1	197.4	12.7
2	2	1	194.7	226.4	116.3	201.0	13.1
2	2	2	389.4	443.2	113.8	200.1	13.5
3	2	1	194.4	219.0	112.6	199.2	13.9
3	2	2	388.8	447.9	115.2	198.4	14.2
4	2	1	194.4	261.3	134.4	198.4	14.1
4	2	2	388.8	439.9	113.2	197.8	13.9
1	3	1	195.6	202.6	103.62	198.2	10.3
1	3	2	391.1	387.1	99.0	195.9	10.35
2	3	1	195.1	195.4	100.1	193.1	11.2
2	3	2	390.2	437.0	112.0	193.4	11.7
3	3	1	195.4	200.3	102.5	195.0	10.1
3	3	2	390.7	378.3	96.8	195.7	11.1
4	3	1	195.3	221.5	113.5	203.1	11.0
4	3	2	390.5	431.8	110.6	202.4	10.9

Appendix E. Autocorrelation of the methane production time series estimated by the climate respiration chamber (CRC) and cubicle hood sampler (CHS) methane for each cow.

Table A.4. Autocorrelation between the methane production rates estimated for each cow by both monitoring devices (climate respiration chamber, CRC, and cubicle hood sampler, CHS), and with a lag of 1. Mean and standard deviation (sd) are presented across cows per monitoring device.

Cow	CRC	CHS
364	0.397	0.193
720	0.609	-0.072
735	0.481	-0.296
769	0.570	0.146
960	0.099	0.006
1345	0.428	-0.237
1369	0.593	0.160
1446	0.357	0.590
1490	0.452	0.118
1498	0.324	-0.089
1516	0.213	-0.491
1517	0.216	0.084
1520	0.673	-0.389
1542	0.334	-0.139
1558	0.499	-0.230
1565	0.344	0.628
1573	0.204	0.070
1948	0.325	0.025
1982	0.235	-0.178
1988	0.320	0.010
2007	0.483	0.025
Mean	0.388	-0.003
sd	0.148	0.268

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Appendix F. Confusion matrices of the true/false positives and negatives classifications estimated by the climate respiration chamber (CRC) and the cubicle hood sampler (CHS) when comparing each cow to the mean methane production level of their population.

In addition to the data analysis presented in the core of the text, we conducted an analysis aiming to evaluate the ability of the cubicle hood sampler (CHS) to determinate if the methane production level of one cow is above or below the mean level of its group. This information is relevant when selecting cows based on their lower methane production rate (MPR) in comparison to a specific population, for example when genetically selecting animals.

For this, we compared the classification (above or below the mean level) defined by the CHS and the climate respiration chamber (CRC), with and without using the hierarchical methane rate (HMR) model.

A mean MPR level was calculated for each dataset. This resulted in five mean values: one for the original CRC set (ORS), the original CHS set (OHS), the CRC subset (ORS subset), the CHS subset (CHS subset), and the CHS training set (HTS). Then, the mean MPR of each cow was classified as being above or below the mean MPR level of its population. The categorizations defined by the two devices for each of the scenarios were then compared.

Individuals that were classified by both devices as being above (or below) their mean population level were labelled as true positives (or negatives). Conversely, the individuals that were categorized as above the population mean by one device and below by the other were labelled as false positives or negatives. Using these labels, the accuracy, sensitivity and specificity were calculated for each case. It gave an indication on the level of correct classifications made by the CHS for the true positives (sensitivity), the true negatives, and the combination of both (accuracy). The outcomes were summarized in three confusion matrices (Figure A.1, Figure A.2, Figure A.3).

The confusion matrix classifying the MPR levels of ORS and OHS against their respective population mean resulted in levels of accuracy of 0.619, sensitivity of 0.583, and specificity of 0.667 (Figure A.1). The two matrices comparing the ORS subset to 1) the OHS subset, and 2) the HTS gave the same results as they both classified cows identically. They thus both yielded accuracies of 0.636, a sensitivities of 0.500, and a specificities of 0.750 (Figure A.2, Figure A.3), showing no difference with or without using HMR with this data. We can note that in all cases, most of the errors were linked to false positives.

Overall, these results showed that the CHS can accurately predict 61.9 to 63.6% of the true and false positives, *i.e.* evaluate if a cow is truly above or truly below the mean methane production level of its population. These results are in line with the ranks obtained with Kendall W, and make the CHS as a promising tool for selecting animals on the basis of their MPR and comparatively to the levels of their population.

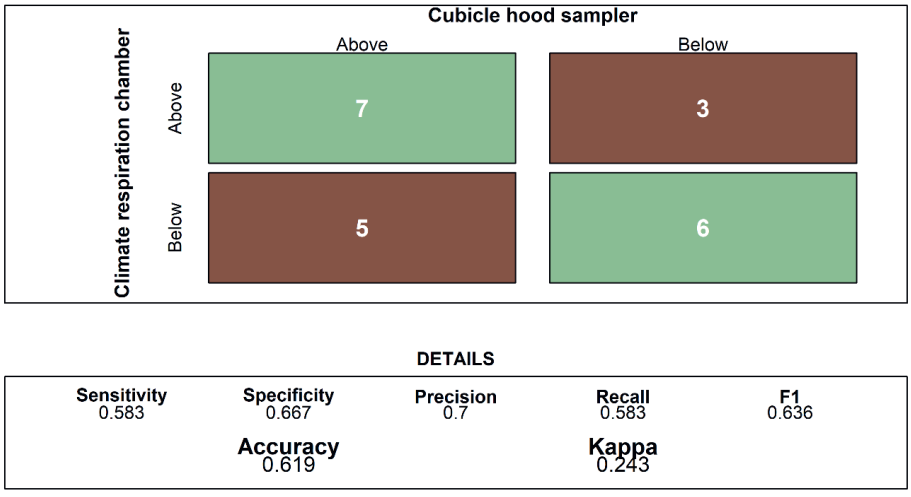

Figure A.1. Confusion matrix showing the classification of the individual methane production rates, as estimated by the climate respiration chamber (CRC: ORS) and the cubicle hood sampler (CHS: OHS). This matrix includes 21 cows.

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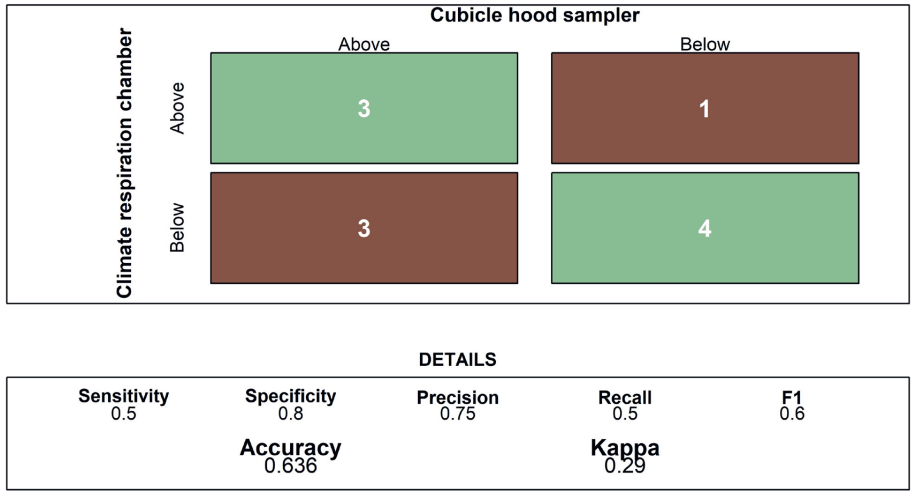

Figure A.2. Confusion matrix showing the classification of the individual methane production rates, as estimated by the climate respiration chamber for the 11 cows subset (CRC: ORS subset) and the cubicle hood sampler (CHS: OHS subset). This matrix includes 11 cows.

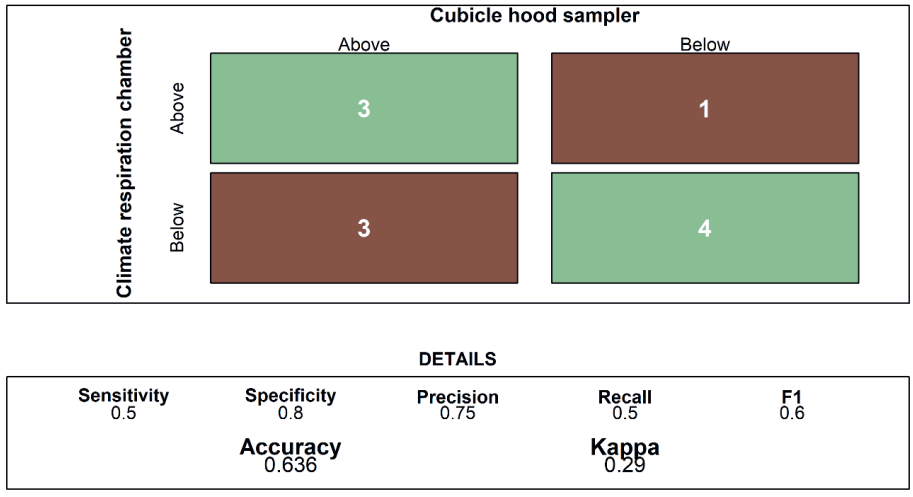

Figure A.3. Confusion matrix showing the classification of the individual methane production rates, as estimated by the climate respiration chamber (CRC: ORS subset) and the cubicle hood sampler after fitting its data with the hierarchical methane rate model (CHS: HTS). This matrix includes 11 cows.

Appendix G. Population and individual scale factor variation from the hierarchical methane rate (HMR) model fit using cubicle hood sampler data.

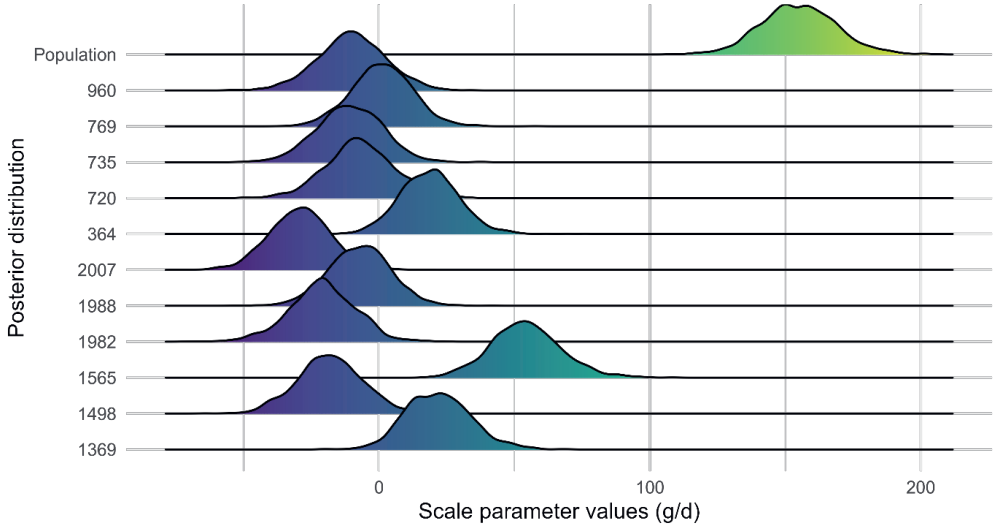

Figure A.4. Ridge line plot of the population scale factor ($\bar{\alpha}$) and the individual deviations from the population per cow (α), indicated by their number (n = 11).

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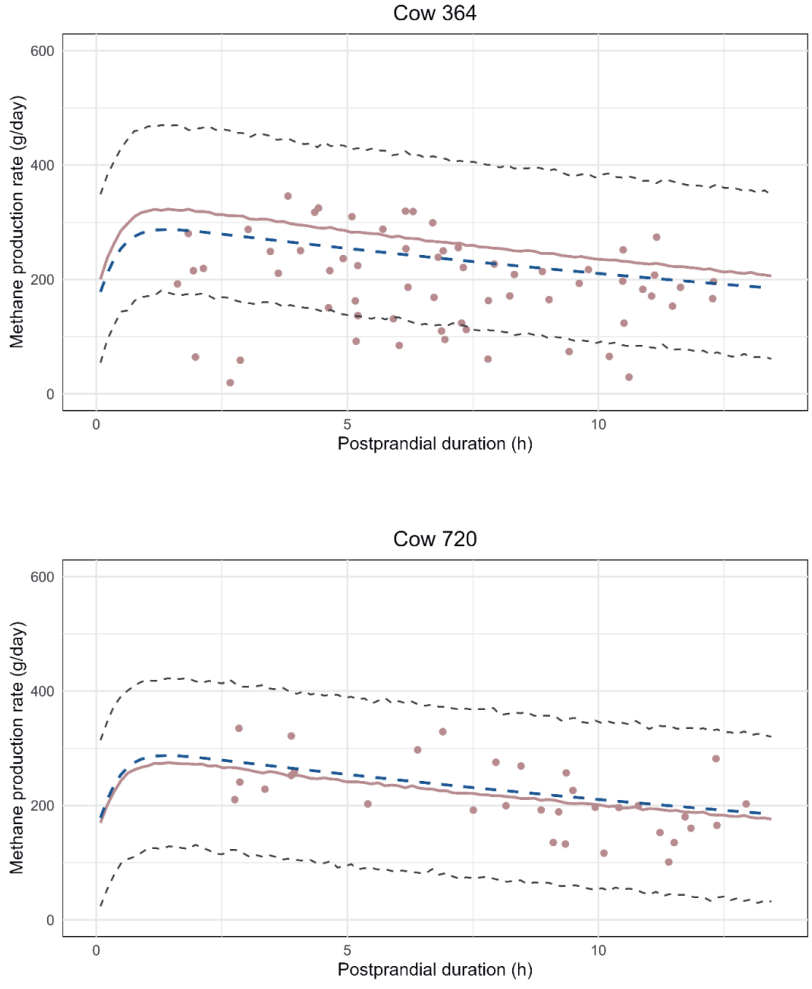
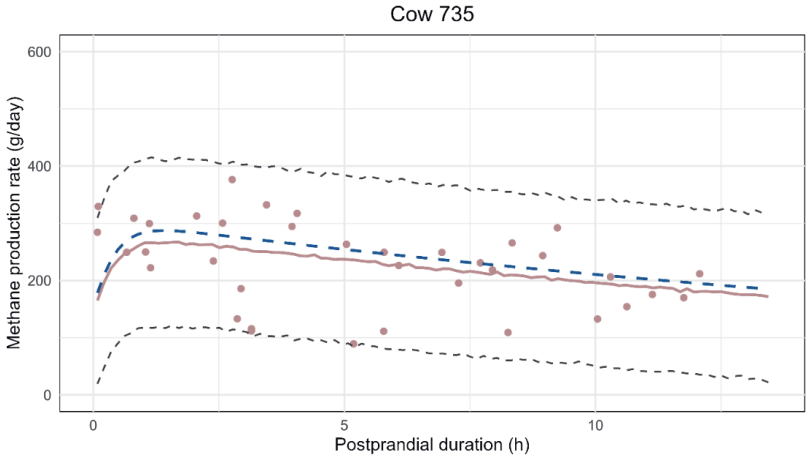
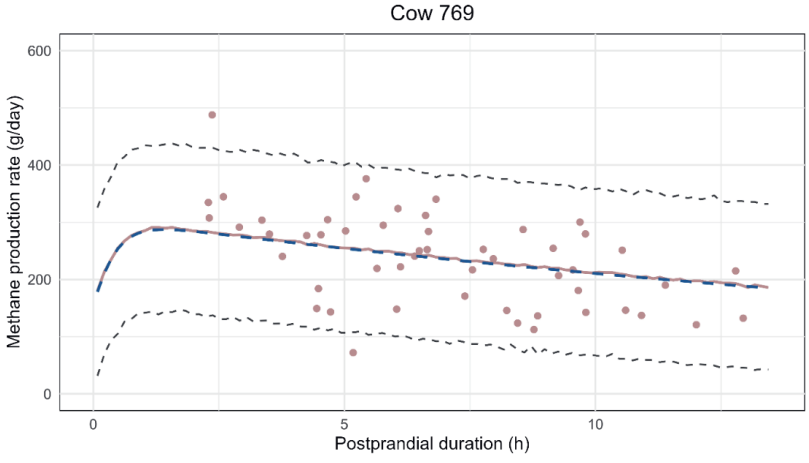
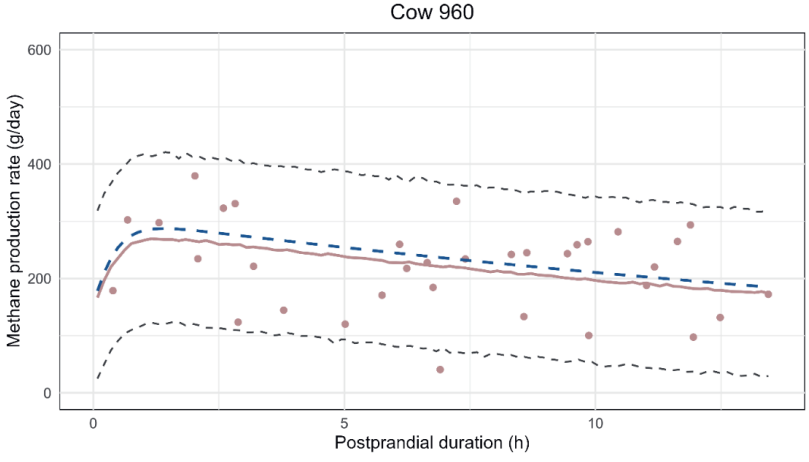
Appendix H. Individual methane production curves fitted with the hierarchical methane rate (HMR) model for the 11 cows of the HTS

Figure A.5. Individual methane production curves fitted with the hierarchical methane rate (HMR) model for the 11 cows of the cubicle hood sampler (CHS) training set (HTS). Blue line: population methane production rate (MPR) curve; pink line: individual MPR fitted curve; dashed lines: credible interval; pink dots: CHS observations.

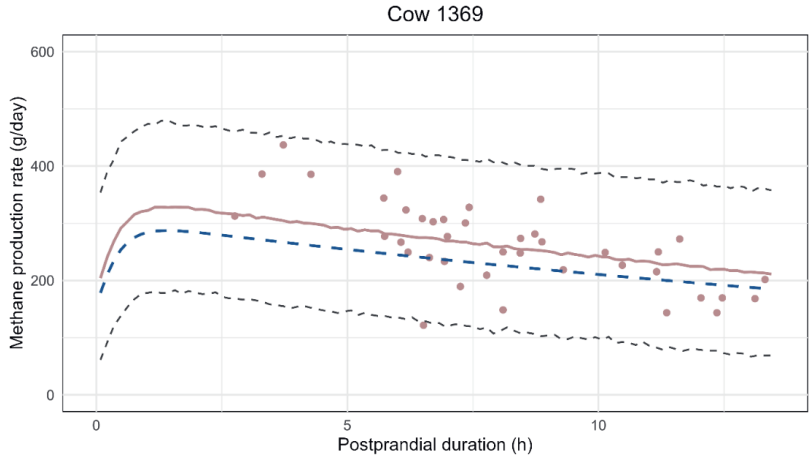


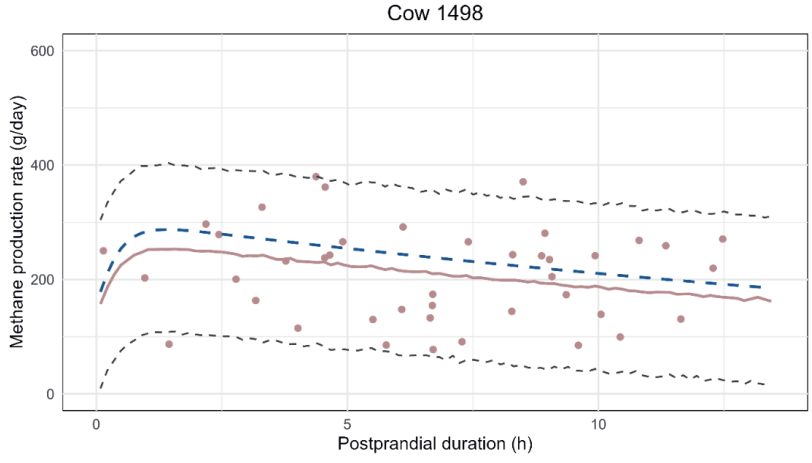

Cow 735
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)

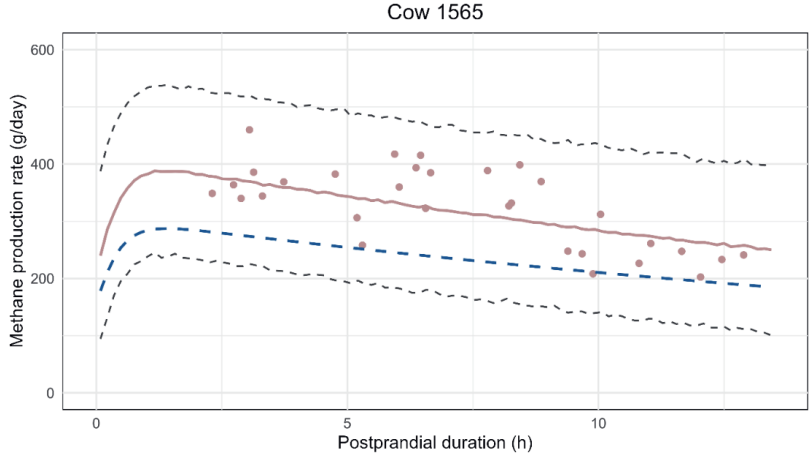

Cow 769
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)

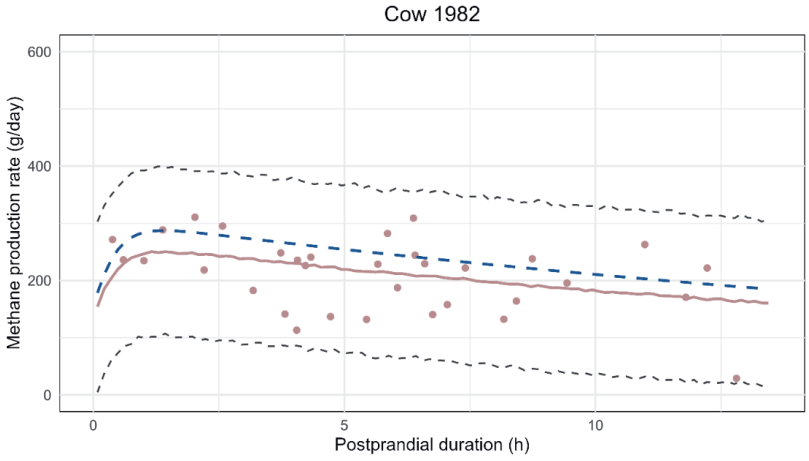

Cow 960
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)

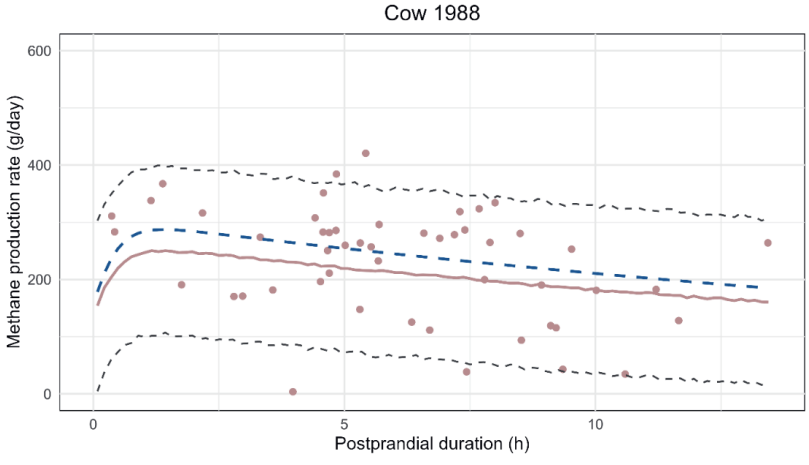
Suppl.

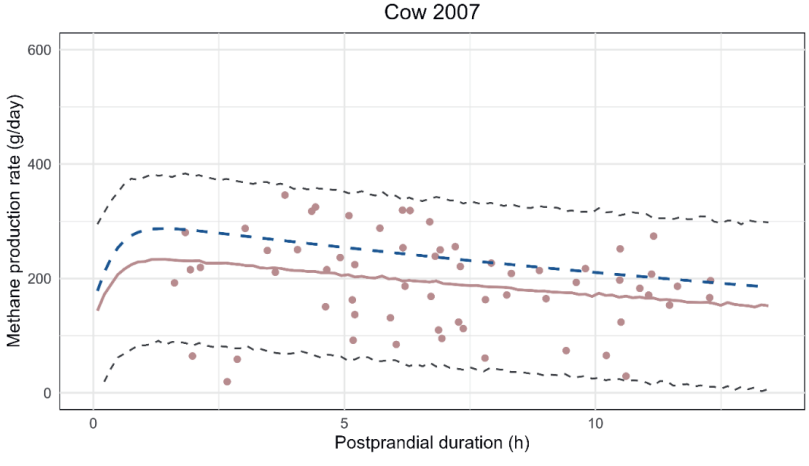

Cow 1369
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)


Cow 1498
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)


Cow 1565
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)


Cow 1982
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)


Cow 1988
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)


Cow 2007
Methane production rate (g/day)
600
400
200
0
0
5
10
Postprandial duration (h)

Suppl.

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Chacha, what a road it has been since we first met for a hot chocolate on day 1 of the university to now. Despite the distance (and with all our moving around, it has been quite some!), we always managed to stay very close and present for each other. A day rarely goes by without us talking, and I deeply thank you for being there for me at all hours of the day and night, all stages of the PhD, to help me out and cheer me up. You are a wonderful, shiny person, and you should never change that. I can't wait to come and visit you in Ivory Coast to discover your new life and celebrate this milestone with you! Ma petite **Clem**, you're a true confidante. How to summarize the range and levels of complicity and mononeuronness we share? With you I have laughed so freaking much. I will always keep very fond memories of our escapades together, that they were the drinking Kastel Rouge at the bars, the sleepless talking nights, or the hikes and camping in the mountains. I love how you are both a true, loving, and supportive friend, but also a teasing and poking one. With you, I have never stopped having fun. Thank you also to **Romane**, **Louise**, **Hélène**, **Mathilde**, **Emeline**, and **Claire** for all the good years, laughs, and tears. Unfortunately, life made it in a way that we went from being together every day to only meeting a few times per year. Yet, knowing you are there always made things feel easier. I believe that what matters in old friendships is to remain present for the important moments, such as today. So thank you for being you, for the joy and support you have always brought me, and for still being here after all these years. I am so glad we met.

Louloute and **Clément** for multiple years you have been our French friends in the Netherlands, bringing to Hadri's expat life and to mine moments that can only be shared with those who truly understand you. In your respective ways, you have brought me love, strength, and support during the PhD. **Louise** I will always admired your passion and devotion to your work, which you are amazing at. Of course not only that, your goofiness, friendliness, and care make being around you a real pleasure. **Clément**, you show what a truly carrying person is. Thank you both for being my friends, from Leeuwarden to Wageningen, Lannion to Paris.

Talking about great friendships, I cannot not mention **Maxime**, **Pierre**, and **Alexandre**. **Maxime** (aka Bichette) you and I started the Dutch adventure together, and you quickly became much more than a friend, feeling more like a member of my own family. You are someone to whom I can tell everything, knowing that I will always get your help and support. You truly and genuinely care about other people which, coupled to your enthusiasm and hecticness,

simply feel great and uplifting. Another truly good person is you chouchou (**Pierre**). You have always made me laughed so freaking much. What I love about you the most is that if I would ever fall on the ground and hurt myself, you are the type of friend that would first laugh at me real hard, and then help. And you **Alexandre**, the artist behind all of the gorgeous illustrations of this thesis. Saying that you are an amazing, good, devoted, funny, and overall simply great friend would be an understatement. Thank you so much for being my friend all these years, for always being there for me, and for putting up with my annoying and endless complains about the illustrations with patience and professionalism. You really nailed it.

I also want to thank my family for always being there for me. A special thanks to my cousins **Claire** and **Marine** for all the good and fun moments we have shared through the years, from growing up as kids to being adults, and for coming to the Netherlands to share this very special day with me. Thank you also to the family of Hadrien, and especially to **Christine**, **Eric**, **Célia**, and **Grégoire** for joining me on this important day.

To my special half, **Hadri**, goes my deep recognition. By now, I have already known you for more than 10 years, and in many ways we have grown together to become the young adults that we are now. I will never thank you enough for being there in all parts of the process of this thesis, from the very first to the last day, in the goods and in the panics. You have helped me shaping so many little details to big choices of my PhD, going from debugging my codes, to discussing what sensors to use, or simply (but it felt like a crucial choice back then) which colours to choose for the ultimate plot of my PhD thesis. Overall, I just simply cannot dissociate this adventure from you. So thank you for being my family in the Netherlands, for living the crazy smelly ferrets lovers life with me, for always wanting to do better, and of course for accompanying me in every step of the way. Doing it without you would have been a completely different story, and I am glad that it went exactly this way.

And then finally, **papa**, **maman**, those to whom I simply owe everything. **Papa** thank you for accompanying me so much through all the phases of my life, and especially for being so helpful during my studies, path decision making, and early career choices. Without you, I would not even have known which studies to pick, nor would have I resisted to the change of format and learning methods between high school and university. You have always helped me finding the right solutions, the right methodologies, the right path, and this not only with patience and love, but also always in an innovative and clever manner. Growing up (and this is still true today as I believe I am still growing up; clearly not in size though), you have given me a model, someone to look up to, on whom I could always rely whenever needed. With you, I have broadened my

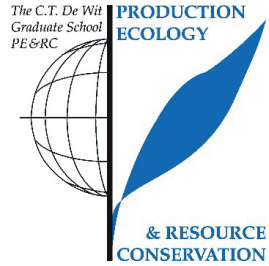
horizons, being stimulated by all the fun and diverse trips, sports, and activities we always did together. You have really stimulated me. I have the feeling that both in terms of personal tastes and interests, but also of career path, the apple has not fallen far from the tree, and I am proud to be, in my own way, following your footsteps. **Maman** pour toi, voici la seule section de cette thèse qui sera en français. Merci pour tout l'amour et le réconfort que tu as toujours su m'apporter. Jamais je n'ai eu à douter que tu m'accorderais ton soutien, ce qui m'a permis de faire toutes sortes de choix, d'expériences (et de bêtises parfois !) en sachant que tu serais toujours de mon côté. Merci de t'être battue au quotidien pour m'offrir une vie agréable, de toujours avoir su m'écouter, et de toujours déborder de gentillesse quoi qu'il arrive. Tu m'as appris qu'on pouvait être une femme forte et indépendante tout en continuant à pleurer devant des dessins animés, à apprécier les belles lumières, et à prononcer beaucoup trop de mots du quotidien de la mauvaise (mais plus drôle) manière. Pour moi, s'il y a une wonder woman sur Terre, c'est toi.

About the author

Cécile Levrault was born on March 31st, 1995 in Montpellier (France). In 2018, she obtained an agricultural engineering degree from the Institut Supérieur d'Agriculture (Lille, France) where she specialised in livestock production. During her studies, she took part in an Erasmus exchange program that took her to the Netherlands for the first time (Van Hall Larenstein, Leeuwarden). She then went to Lyon to specialise in livestock, environment, and health (Institut Supérieur d'Agriculture et d'Agroalimenaire Rhône-Alpes, Lyon, France). After that, she came back to the Netherlands to conduct a master thesis on the lying behaviour of cows at Wageningen University and Research.

She started her PhD thesis on the practical monitoring of enteric methane production from individual ruminants in 2019 as a part of the Agricultural Biosystems Engineering Group of Wageningen University and Research. Since mid-2023, she is working as a postdoc in the same group on topics related to livestock emissions monitoring and precision livestock farming.

PE&RC Training and Education Statement



With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review/project proposal (4.5 ECTS)

Review of the existing practical devices for enteric methane monitoring

Individual assessment of dairy bovines' methane production by an optimized non-invasive on-barn device

Post-graduate courses (5.3 ECTS)

Emission modelling for livestock housing system; Ghent University, Belgium (2019)

Ammonia, greenhouse gas, and aerosol transmission from livestock systems: an interdisciplinary approach on measuring, modelling and mitigation; ATB Potsdam, Germany (2019)

Basic statistics; WUR PE&RC, the Netherlands (2020)

Tidy data; WUR PE&RC, the Netherlands (2021)

Laboratory training and working visits

On-site training by the laboratory staff; Wageningen Livestock Research & Air quality lab Innovatron

Competence, skills and career-oriented activities (7.7 ECTS)

The essentials of scientific writing and presenting; WUR PE&RC (2019)

Project and time management; WUR PE&RC (2019)

Supervising BSc & MSc thesis students; WUR PE&RC (2020)

Ethics and animal science; WUR WIAS (2020)

Making impact: increasing the relevance of research through science society interaction; WUR PE&RC (2021)
Communication with the media and the general public; WUR PE&RC (2021)
Career perspectives; WUR PE&RC (2023)

PE&RC Annual meetings, seminars and the PE&RC retreat (1.2 ECTS)
PE&RC Day (2021, 2022)
PE&RC Last year retreat (2022)

Discussion groups/local seminars or scientific meetings (5.5 ECTS)
Emissions and nutrient management (2021)
International symposium on dairy cattle nutrition: energy metabolism in dairy cows (2021)
Eragas breakfast clubs (2022)
Alternative system for practical measurement of dairy cows' methane production rates for the CCC farming Rennes (2022)
ECPLF Vienna (2022)

International symposia, workshops and conferences (6.7 ECTS)
Agricultural Engineering (AgEng) Conference; oral presentation; Berlin, Germany (2022)
Non-CO_2 Greenhouse Gases (NCGG) Conference; oral presentation; Amsterdam, the Netherlands (2023)
Channel Network Conference (CNC); oral presentation; Wageningen, the Netherlands (2023)
European Federation of Animal Science (EAAP) Conference; oral presentation; Lyon, France (2023)

Societally relevant exposure (0.3 ECTS)
Comic “There is something about ammonia” for primary school students (2021)

BSc/MSc thesis supervision (12 ECTS)
Detection of cows’ head position using ultrasonics sensors for methane measurements
Optimisation of background methane measurement strategy of the Cubicle Hood Sampler
Validation of radio frequency identification as an indicator of the cows’ head position within the Cubicle Hood Sampler
Pose estimation of cows in a methane monitoring unit using a key point detection algorithm

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