AARHUS UNIVERSITY

Internship at SEGES Innovation P/S

Determination of Methane in Practice

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Abstract

This project is based on a literary analysis and a data analysis with data collected from a Bovaer herd trial in Assendrup, performed by Aarhus University Foulum and SEGES innovation P/S. The subject to the project was defined in cooperation with SEGES and supervised by Nicolaj Ingemann Nielsen, Head consultant at SEGES and Christian Friis Børsting, Senior consultant at Aarhus University, Foulum.

The literary comparison between the Sniffer and GreenFeeder highlighted the large capacity of the Sniffer, and the suitability for trait-screening. Behavioral and technical biases regarding the spot-sampling methods were highlighted, to point out which objects that must be considered before a trial. The results of intensive literature comparison show, that the GreenFeeder has a higher correlation with the Respiration Chamber, which is considered the golden standard, and is thereby an indication of a more precise method than the Sniffer. Furthermore, an international study showed that the Sniffers' models generally underestimated the methane production found in the Respiration Chambers.

The results of the data analysis also found the GreenFeeder to be more precise than the Sniffer. Furthermore, it indicated that the Kjeldsen- and Pedersen-models estimated a higher CH_4 production than Madsen, which appeared higher than the GreenFeeder. A ranking of the 66 cows into three quartiles (Low- Medium- and High-emitters) were performed, where no more of 50% of cows in one quartile of the GreenFeeder were retrieved by the Sniffers' models. An explanation of the high disagreement between the systems could be due to a behavioral bias of individual cows in the GreenFeeder, which could affect the results into wrong directions.

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1 The internship at SEGES P/S

My internship as SEGES took place in the autumn of 2022 from September to December. Here I had regular visits every week. SEGES is a private non-profit research- and development company, which helps Danish agriculture to maintain competitiveness in a rapidly changing world. The overall company has app. 530 employees that deals with agriculture from all perspectives. The department that I have been connected to is the largest department of SEGES' livestock departments; Health and Production. Here there are 27 employees associated, including me, counting: Consultants, laboratory staff, veterinarians, and student assistants. My advisor, Nicolaj Ingemann Nielsen, Head Consultant, belongs to this department.

1.2 SEGES accomplishments

The assignment of the internship was to be handed in the 21st of December. I was provided a student's office in the department, wherefrom I could follow ongoing activities in the company that was going on. These activities include both coffee-chats, meetings, lunch-conversations etc. I experienced these informal chats to be very important for the work and personal environment in the company and for myself.

1.2.1 Relevant activities

Throughout the semester I went to different activities that represent some of the tasks that an employee at the department has to cover. I went on a few *Farm visits*, both the 15/9 and 7/11 at Koldkærgaard and Fjerritslev, respectively. In Koldkærgaard, the purpose of the visit was to collect and assemble a GreenFeeder. Furthermore, the farm had to be inspected more thoroughly to detect possible problems regarding the GreenFeeder installation in the barn. In Fjerritslev, Nicolaj Ingemann and I, inspected 2 already installed GreenFeeders, which had to be serviced. Furthermore, samples from roughages and concentrates were collected. This gave me an insight into the importance of being able to collect representative samples, where neither the cattle had selected preferred parts of the feed mix, nor where the weather had had an impact to the dry matter content of the feed.

On the 13th of October I went to the *Laboratory* of SEGES, where I tried different laboratory techniques for feed analyses. Here, again, the importance of homogenous and thereby representative collection of the samples shined through. I got insight into how the different feed mixtures get grinded into a form, wherefrom its nutritional composition can be analyzed. I was also helping with registration of results with respect to the specific trials.

SEGES also presents its work on different congresses, focusing on both farmers and consultants. I went to the *Feeding Day* in Herning the 30th of August before the beginning of the internship. Presentations of how to increase protein efficiencies in dairy cattle, the protein feed resources of the future, as well as how to reduce methane through feed additives etc., were presented. Thereby, agricultural stakeholders have an opportunity to gain insight into how agriculture could develop in the future and contribute with inputs to the work and results of the different projects.

On the 27th of September, I went to Skanderborg together with Martin Øvli Kristensen, Frederikke Hahn Lau-Jensen and Nicolaj Ingemann Nielsen to join a *Consultant Educative incentive* by SEGES. Consultants came for a 3-day cattle-advising course, where they were equipped with tools to evaluate and discuss the imminent climate initiatives. They were taught about the ESGreen-tool, which calculates the climate footprints of a specific farm through energy, import and export. Expert presentations about the effect of feed additives on CH_4 etc., were held, which were discussed with practical perspectives in plenum. Through these discussions I got an awareness of the practical obstacles that might appear when climate actions are to be implemented. Also, I got a view on some of the issues that the consultants face when they are to convince farmers about the implementations and future strategies.

On the 11th of October I went to *Aarhus University, Foulum* to meet with Christian Friis Børsting and some of his colleagues to discuss issues related to the use of GreenFeeder for CH_4 measurements. As they have great expertise in handling of cattle, as well as the use of different equipment (Respiration Chamber, Sniffer, GreenFeeder etc.), I got a great input on where sources of error can occur and how to avoid them.

I went to one of the monthly *departmental meetings* on the 18th of November. The employees got reminded about deadlines, briefed about the focus areas and the overall strategy of SEGES. An overview of the financial costs of the projects, related to the fundings, were presented to open the discussion of the function of SEGES in general and what the outputs of SEGES are meant to be. This discussion included the optimization of the synergy of the department and how to put the overall multidisciplinarity of the department into play to ensure the best possible output. Also, presentations were made for the department to gain insight into the progress of project. These presentations were informal but professional, from both inland and international perspectives informing the colleagues about vastly different issues from apart those encountered in Denmark.

2 Introduction

Methane (CH_4) is a potent greenhouse gas that accounts app. 16% of the total Anthropocene greenhouse gas emissions in 2020. The largest contributor is the enteric fermentation from livestock production which has a share of app. 29,5% of the global CH_4 emissions (*Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2020.*, 2022). Thus, enteric CH_4 accounts for around 5% of the total Anthropocene greenhouse gas emissions. According to Eu and UN climate protocol, DK has to reduce emissions and therefore, there is an incentive for the Danish government to have an aim of reducing the emitted greenhouse gasses from the agriculture with app. 7.1 mio. tons of CO_{2e} in 2030 (The Danish Government, 2021). This program estimates a reduction potential of 1 mio. tons of CO_{2e} in the enteric fermentation of ruminants, by the addition of feed additives in 2030.

The need for CH_4 reducing feed additives is accompanied by the need for precise and accurate methods to verify the effect. Therefore, various CH_4 measuring methods have been developed to be able to measure the production of CH_4. These methods can serve different purposes, such as determining the absolute level of CH_4, a certain change in CH_4 or simply a ranking of individuals emitting CH_4. The properties of each method must be considered in the planning process, by evaluating the scopes of application and the results obtained in other studies. However, the properties of more than one measuring method might fit the objectives of a trial, which thereby complexes the selection process.

In the “Measuring and reducing methane in Practice”- (METAKS-) project of SEGES Innovation, the framework focuses on credible data from large production trials, which emphasize precision, replicability and applicability to the method of choice. This is to be able to accurately quantify the effects of feed additives in cattle herds, to investigate the potential in various breeds, farm systems and different additives.

The methods of relevance for this trial are the Sniffers’ system and the GreenFeeder (GF). The aim of this study is to describe and specify differences between the two measuring methods by a literary review and a data analysis of a specific farm trial involving both methods.

3 Measuring methods in practice

The Sniffer is a spot-sampling system, which with its ability to be installed into Automatic Milking Stations (AMS) as well as feed bins, is a non-invasive and cost-effective system. An illustration of the system is showed in Figure 1.

Figure 1: Sniffer equipment at Assendrup. Gas tube installed in feed through (Left) connected to the AMS (Right).

The system is able to measure concentration of CO_2 and CH_4 in the exhaled gas from the cow while it is being milked, which with a metabolic model is able to quantify a CO_2 and thereby a CH_4 production (Haque et al., 2014).

The metabolic model is determined by the CIGR-report (Søren Pedersen et al., 1984). The report describes the Heat Production (HP) of cattle through the metabolic rate of an animal's basic maintenance that varies with the physiological state and properties as described in equation 1:

$$eq\ (1): HP\ (watt) = 5.6 \cdot kg\ BW^{0.75} + 22 \cdot kg\ ECM + 1.6 \cdot 10^{-5} \cdot number\ of\ days\ in\ pregnancy^3$$

The model expresses the heat production as a function of bodyweight, energy corrected milk yield and the number of days in pregnancy. Some exceptions can be found as the metabolism varies in practice. For instance high yielding cows are mobilizing a lot of fat, which results in a relatively lower heat production (Storm et al., 2012).

From the heat production, the CO_2 production can be determined in two different ways, giving two somewhat distinguishable results. (S. Pedersen et al., 2008) made a model describing the CO_2 flux, as shown in equation 2:

$$eq\ (2): CO_2\ production(l\ day^{-1}) = 180\ (l\ CO_2\ HPU^{-1}\ hour^{-1}) \cdot \text{HPU} \cdot 24\ (hours),$$

where the Heat Production Units (HPU) is HP (KJ) divided by 1000 watt.

Madsen et al., 2010, made another desribtion of the CO_2 production, as shown in equation 3:

$$eq\ (3): CO_2\ production\ \left(l\ day^{-1}\right) = \left(\frac{HP\ (KJ)}{21.75\ KJ\ \ l\ CO_2\ produced^{-1}}\right) \cdot 3600(seconds) \cdot 24(hours)$$

The two models have different approaches. Pedersen et al., 2008, estimates a volume of 180 L CO_2 is the volume produced by dairy cattle per hour, given by the specific heat producing properties of the cattle. Madsen et al., 2010 estimates a heat production of 21.75 KJ per liter CO_2, given by the specific heat producing properties of the cattle.

However, the credibility of both approaches can be criticized on their estimates, on which the models are based, which will be further discussed.

The CH_4 flux is then converted from CO_2 by equation 4 (Madsen et al., 2010):

$$eq\ (4): CH_4\ production(g\ day^{-1}) = CO_2\ estimated\ production\ (l\ day^{-1}) \cdot ([CH_4]:[CO_2]) \cdot 0.714(g\ l^{-1}),$$

where 0.714 is the density of CH_4, which can convert the volume of the gas into mass for the sake of convenience when comparing with the GF data.

The GreenFeedTM (C-lock Inc., USA) is also a spot-sampling system, which is supposed to mimic an ordinary automatic feed bin. An illustration of the system is showed in Figure 2**.**

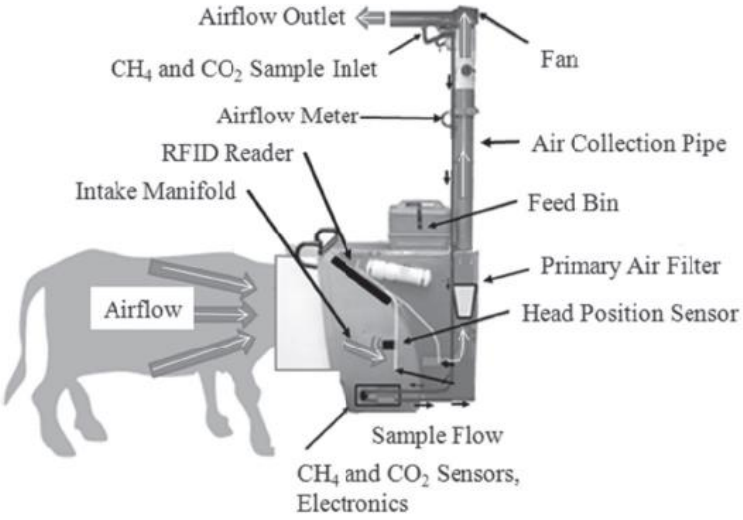

Figure 2: Layout of the GreenFeed system. RFID reader = Radiofrequency identification. Source: Huhtanen et al., 2015

The system attracts the livestock by bait, in the form of concentrates, wherefrom the measuring procedure begins. This method is more automatic than the Sniffer, in the sense that it does not use mathematical assumptions, which can be associated with uncertainties. The omission of these notations can be done, as the system is equipped with a combined animal detector-, flowmeter and infrared air gas analyzer (Jonker & Renand, 2020), which can detect CH_4 production directly, without the use of a CO_2 conversion ratio nor the metabolic models.

The procedure begins with the livestock being attracted to the system with pelleted concentrates, wherefrom the system can detect the individual through ear tag recognition. A detector then determines the distance from the cow to the fan, which drags the exhaled air into the system. The head-detector determines whether the distance between the head and the inlet is too far for the sample to be approved. The flowmeter then determines the volume of exhaled air, and the infrared gas analyzer emits light in a wavelength that matches the molecule of interest (Huhtanen et al., 2015). Afterwards, a detector detects how much of the emitted light that is absorbed by the molecules, which is a measure of the concentration of gasses. The CH_4 flux in the system is determined using the principles of volumetric flow rates of gasses, as described in McLean & Tobin, 1987, and illustrated in equation 5:

eq (5): $$CH_4\ flux(l\ min^{-1}) = [CH_4]_{Exhaled}(cm^3\ m^{-3}) - [CH_4]_{Background}(cm^3\ m^{-3}) \cdot Drygas\ flux\ (l\ s^{-1})$$

The dry gas flux is a measure of the gas' flux through the pipe. This accounts for the dimensions of the pipe and the velocity of the gas. The background concentrations should be stable before being used. Thus, the background concentrations are determined using ambient air concentrations before and after the visit of a cow when the gas concentrations stabilize, as described in Huhtanen et al., 2015. The overall treatment of data is done through the software of C-lock inc., meaning that the data provided by the GF has already been "manipulated" roughly. This includes the deletion of invalidated measures from cows being too far from the inlet, as well as the processing of electro-units expressed by the gas analyzer, to the mass units that are provided. Thus, it is relatively easy to operate the machinery, but as it is impossible to gain insight into the software, we do not know exactly how the data has been processed.

4 Analysis and discussion

The Sniffer and GF have different scopes of application. Therefore, before the installation of a specific measure to a farm, it is important to consider the properties of method and farm, to evaluate the advantages and disadvantages that accompany a specific method.

4.1 Method properties

The scope of application should fit the description of the project of interest. Thus, various parameters are worth considering. This includes financial costs, animal availability, replicability, uncertainties, implementation options etc. In this section I will examine the two spot-sampling methods and their applicability in an ordinary farm.

4.1.1 Statistical variation and uncertainties compared to Respiration Chamber systems

Since it is very inconvenient to measure with both the Sniffer and the GF at the same time, many studies compare results to data obtained in respiration chambers (RC)'s. Respiration chambers are closed chambers in which it is possible to control many external factors, as well as the changes within the chamber. The dimensions of the chamber are known which makes it possible to recon the change of concentration of the various gasses. The system has an inlet of air with known content, and a pump connected to the outlet, which leads to measuring sensors.

The outlet of an RC should be equipped with overly sensitive sensors in order to determine the gas production of the cow, as well as the airflow, with a very high certainty. Due to the reliability and the fact that it is convenient to control the environment inside the chamber, it is considered being the standard reference method for estimation of CH_4 in ruminants (Storm et al., 2012).

Certain sources of error should be considered to keep the level of variance low in the RC. These errors include: Ducting efficiency, analyzer error, mix of air inside chamber, and exclusion of extraneous CH_4 which typically enters during feeding and milking (Hellwing et al., 2012; Hristov et al., 2018).

A meta-analysis from New Zealand, analyzed four methods' abilities to determine CH_4 emissions in relation to dry matter intake (DMI) in dairy cattle (Jonker et al., 2019). Figure 3, illustrates the weighted residuals of the analysis, highlighting the low variance observed in an RC, of up to 4.5 times lower, compared to other CH_4 measuring methods.

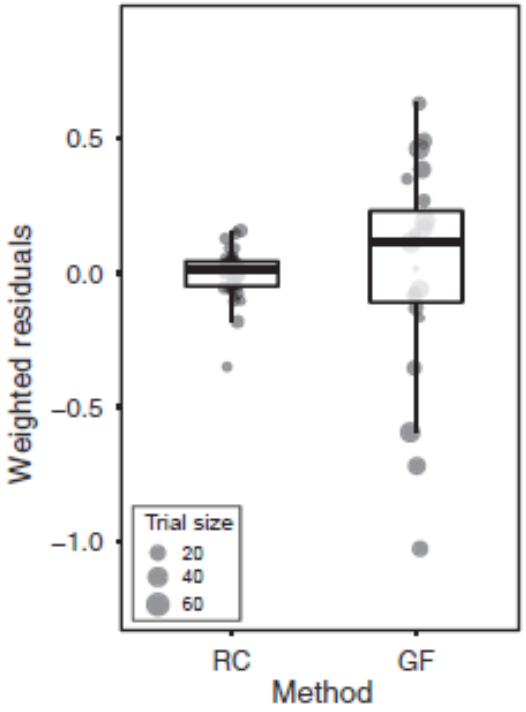

Figure 3: Weighted residuals (Square root of Observed-Predicted) of number of animals that make up a treatment mean with the use of two measuring methods; Respiration Chamber (RC) and the GreenFeeder (GF). The figure is modified to maintain relevance. Source: Jonker et al., 2019

Difford et al., 2018, assessed a ranking between 10 lactating Jerseys and 10 lactating Holsteins on individual level, where results of the same herds from the Sniffer and RC were compared. This was to correlate the CH_4 production with a genetically oriented point of view. The correlation between the predicted values of the Sniffer technique related to the measured values of the RC. The live weight, ECM and DMI were retained as control variables, which had very similar descriptive statistics in terms of variance and repeatability (CV% and t). Comparing the Sniffer and the RC on standard deviation, the measured CH_4 production was with means of 573 and 521 L CH_4 day^{-1} (~9% difference), associated with deviations of 73.9 L CH_4 day^{-1} and 56 L CH_4 day^{-1}, for the Sniffer and the RC, respectively, which is indicated in the individual-level correlations. The variance for the methods were thus of ±12.9% and ±10.7% for the Sniffer and RC, respectively.

In comparison, Velazco et al., 2016, compared CH_4 production by the use of GF and RC. This study was performed in a comparable basis, as the experiment of Difford et al., 2018, with precautions in terms of keeping the measured herds of a relatively low number of individuals (n=10), as well as having a sufficient adaption period. The CH_4 production in the GF and RC were compared in Velazco et al., 2016. The methods measured methane production means of 198.3 and 214.6 produced g CH_4 day^{-1} (≈8% deviation), for the GF and RC, respectively, with a standard error of mean (SEM) of 3.0.

The experiments vary in terms of liveweight, as the individuals of Difford et al., 2018 and Velazco et al., 2016 are having mean liveweights of app. 565, and 365 kg, respectively, reflected in their respective CH_4 production. Thus, the CH_4 production should not be compared between the two

studies, whereas the uncertainties in the experiments remain comparable as they are analyzed in relation to the RC.

A literary review (Huhtanen et al., 2019), compared 6 studies of GF systems with data obtained in RCs (illustrated in Figure 4). A strong relationship was determined (R^2=0.92). The analysis identified the prediction error of the intercept to be mostly due to random variation (88%), which can be partly explained by variation in DMI (≈ 1.2 kg day^{-1} lower in RC than GF) as well as too short measurement periods and too few individuals in the GF. Garnsworthy et al., 2019, reviewed and compared the suitability of measuring methods for genetic evaluations. In this regard, the Sniffer and GF methods were compared to obtain variance components, accuracy and repeatability as illustrated in Table 1.

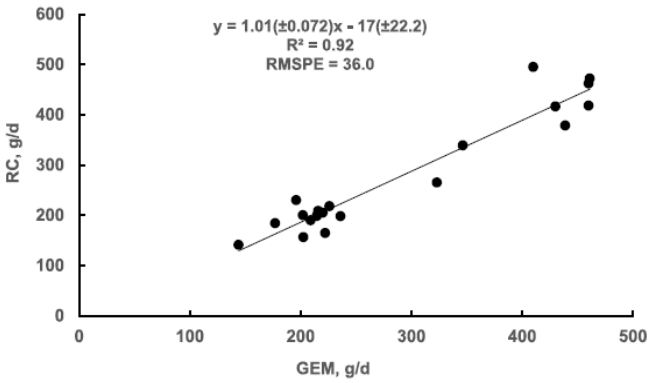

Figure 4: Relationship between GreenFeed emission monitoring system (GEM) and Respiration Chamber (RC) in CH_4 production (n = 20) *Source: Hristov et al., 2018*

Table 1: Comparison of methods for measuring CH_4 emissions in dairy cattle. Only a section of the original table is included to maintain relevance. NDIR CO_2 t1= Nondispersive infrared, output calculated from t1 (BW, ECM, and DIP). Mean SD = Mean daily CH_4 output (liters), CCC = Lin's concordance correlation coefficient. *Source: Gansworthy et al., 2019*

Alternate Methods [1] Versus Respiration Chambers								
Method	N Cows	N Obs	Mean S.E.	Rep S.E.	Between-Cow CV [2]	Total CV	Correlation [3] (S.E.)	CCC [4] (S.E.)
NDIR CO_2 t1	27	63	586 (19.4)	0.59 (0.13)	13.2	17.2	0.64 (0.18)	0.14 (0.19)
GreenFeed	27	63	453 (9.8)	0.75 (0.08)	9.7	11.2		
GreenFeed	27	63	433 (8.7)	0.64 (0.08)	12.8	15.9	0.81 (0.10)	0.41 (0.12)
Respiration Chambers	27	63	459 (6.5)	0.51 (0.09)	8.1	11.3		
NDIR CO_2 t1	20	60	573 (16.8)	0.58 (0.11)	10.1	13.1	0.72 (0.11)	0.38 (0.21)
Respiration Chambers	20	60	521 (13.7)	0.61 (0.12)	9.1	11.7		

The Sniffer is abbreviated as NDIR, which is the instrument used in the gas analyzer of the experiment (Guardian NG from Edinburgh Instruments) installed in AMS. The first row of the table the GF is compared to the Sniffer (NDIR). This row reveals that the GF has a higher repeated measures correlations (0.81 vs. 0.72), which means that the measures in the GF are better correlated to the golden standard, the RC, than the Sniffer. There was generally a lower correlation between the "alternate methods" than when a certain method was compared to the RC. The study concludes that the correlation GF correlation to the RC of 0.81 (std. err. = 0.1) outcompeted the correlation between the Sniffer and the RC of 0.72 (std. err. = 0.11).

The correlations with the RC were estimated to be less than 0.9, as the methods could not be recorded simultaneously, and therefore had to be recorded in cross-over designs, which increase the imprecision. Thus, the fit between the methods might be underestimated. Furthermore, the comparison-study conclude a lower precision in the concentration-based methods compared to methods with mass flux measures.

There is a general congruency that the mathematical assumptions used for predicting values of CH_4 emissions through concentration-based methods, like the Sniffer, are associated with uncertainties, as it is impossible to include all biological parameters in a practical model (Huhtanen et al., 2015; Wu et al., 2018). An amalgamation of an international dataset of measured CO_2 and calculated CO_2 with the use of the Sniffers' equations, by Maria Holst Kjeldsen, AU Foulum, has enlightened whether the use of these mathematical assumptions is sufficient to explain the metabolic rate and thereby the CO_2 production. In Figure 5 the measured CO_2 production is correlated to the modelled CO_2 production. The dataset used is a large (n = 1500) international set, where cows have been measured in RCs. 328 of these cows included the required phenotypic parameters (BW, ECM, and pregnancy). From these cows it was possible to calculate the modelled predictions of both Sniffer models and verify it with the reliable data measured through the RC. The two Sniffer models made by: S. Pedersen et al., 2008 and Madsen et al., 2010 are evaluated. It shows a tendency of a general underestimation as well as a higher deviation, when the cow is expected to produce larger amounts of CO_2. This means that there is a general risk of underestimating the CO_2, and thus the CH_4 production in both models. Especially, the method of Madsen et al., 2010 seems to underestimate the gas production.

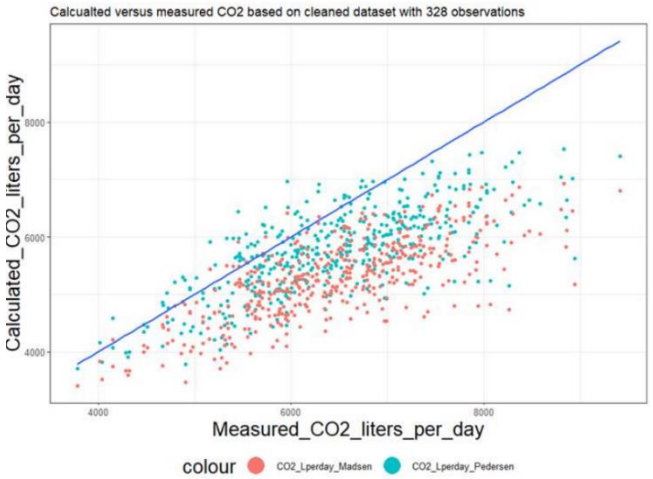

Figure 5: Measured CO_2 production plotted against the calculated CO_2 production (n = 328). Two different methods are used for the estimation of CO_2 production; Madsen et al., 2010 (Red dots) and Pedersen et al., 2008 (Blue dots).

Source: Pers. communication w. Maria Holst Kjeldsen, AU Foulum, 2022.

Madsen et al., 2010 uses 21.75 kJ liter CO_2^{-1} , which is based on an average calculation from a "standardized diet" from (Chwalibog, 1991). It is not specified what the diet consists of. The model of Pedersen et al., 2008, is currently used in the Sniffer, as no better alternative is available. This model is based on three studies examining only 2 cows each, where one of the studies is not even

published (Pedersen et al., 2008, Van Knegsel et al., 2007 and van Straalen et al., 2007). van Straalen et al., 2007, is a conference paper and not a peer-reviewed study, whereas Van Knegsel et al., 2007 is a published paper, which did not specify which types of cows were used, nor their CO_2 production. The models were made with the purpose of determining the ventilation flow in barns (Pedersen et al., 2008), which might explain some of the difficulties of generalizing the HP-unit to an individual level.

As the GF does not use the metabolic rate as a measure of CH_4 production, it can exclude these mathematical uncertainties.

The uncertainties associated with the GF and the Sniffer methods can be determined in relation to the golden standard, the RC, as a measure of precision. The correlation comes with an increased uncertainty, as the methods are not recorded at the same time nor with the same individuals, but in a cross-over design. To be able to make a direct comparison between the GF and the Sniffer, a controlled study analyzing the same individuals, and including an RC as the control reference, would be optimal (Zhao et al., 2020). Generally, studies show different statistical variance for the methods, with advantage to the GF method, reflected in more precise CH_4 production estimation. This could be due to the somewhat questionable quality of estimation in the two models used in the Sniffer.

4.1.2 Pros and cons between the system specifications

When considering which system to choose for a specific task, the system specifications are worth noting, as some conspicuous factors stand out for both methods, which also reflect the associated uncertainties discussed before.

The financial expenses of the systems are distinguishable, as the GF system has an estimated price of around 90,000$ (C-locking.com), whereas an integrated Sniffer system comes with a price of around 20,000$. The difference in price is reflected in equipment integrated in the GF, which is missing in the Sniffer. The most prominent difference is the automatic airflow system with associated sensors, that makes it possible to directly estimate the CH_4 production instead of using mathematical notations to estimate the flux (Jonker et al., 2020).

The GF system is generally equipped with a lot of sensors, both flowmeters, gas sensors (CH_4/CO_2/H_2/O_2), wind sensors, as well as head positioning sensors. The head positioning sensor is able to detect the distance between head and inlet and is thereby validating whether the system is capturing the exhaled air or not (Jonker et al., 2020). When the Sniffer is lacking both flowmeters, wind sensors and the head positioning sensor, algorithms are used to compensate for an eventual

diluted concentration of gases (Pers. communication w. Trine Michelle Villumsen, AU Foulum, 2022). For instance, it has been shown that the standard deviation of measurements increases by increased duration of the cow being in the robot, which is interpreted as an expression of the cow being more willing to keep its head in the feeding through at the beginning of the milking session (Pers. communication w. Trine Michelle Villumsen, AU Foulum, 2022). The lack of head positioning sensor generally makes it difficult to compare between studies, as various compensatory computing approaches are adopted and being used differently across scientific trials.

Thus, the main differences in the fiancial properties between the systems can be explained by the GF system being more automatized, having the equipment to be able to adjust for eventual sources of error, whereas the Sniffer uses mathematical models, which is associated with greater uncertainties.

The test capacities of the systems also differ, reflecting some of the current usage of the systems. When the Sniffer system is installed to an AMS, it can record 40-70 cows, around 2-7 times a day for 7-10 days in order to get sufficient data about CH_4 production. The GF system is recommended to analyze no more than 15-25 individuals per GF unit in a loose housed system, with recordings for app. 7 days (Garnsworthy et al., 2019; Hammond et al., 2015). A Dutch study (Koning et al., 2020) however challenged this, and examined groups of up to around 50 individuals for 2 weeks without impacting the visitation rate nor the standard deviation. This study highlighted the importance of having measurements distributed at all times of the day, to be able to evaluate the test capacity. In van Breukelen et al., 2022, a population analysis for genetic evaluation was performed with both the Sniffer and GF, illuminating the different testing capacities of the methods. The Sniffer recorded 31,579 weekly averages from 1,744 cows, whereas the GF recorded 4,356 weekly averages from 724 cows, from 25 setups for each method. The ability to test a large number of individuals in a relatively short time in the Sniffer system is what makes it an attractive method for large-scale evaluations such as in population analyzes, which will be further discussed. However, when evaluating test capacities of the methods, it is important to keep the visitation-rate and -distribution in mind. These factors vary across experimental designs, farms etc. (Koning et al., 2020).

4.1.3 General biases of spot-sampling methods

Both the Sniffer system and the GF are spot-sampling systems, that comes with the advantages of mimicking the daily routine on a farm, and thereby are non-invasive to the natural behavior in the barn. This way of putting emphasis on the behavior of the analyzed population includes some general challenges, in the form of behavioral- and technical biases, that must be dealt with in order to keep data reliable. Behavioral biases are mostly observed in the GF in terms of uneven temporal and population wise distribution of sampling. It is not guaranteed that the system is visited evenly throughout the day. Generally the system is visited mostly between 07-08 and 13-14, and visited the least in the night between 01 and 06 (Hammond et al., 2015). If the measurements are not successfully distributed at all times of the day, it becomes uncertain when extrapolating the data. Olijhoek et al., 2016, showed the importance of evenly distributed data in an experimental design involving a nitrate treatment (Illustrated in Figure 7). There was only significant difference between the treatments just after feeding. Thus, if cows were only observed after feeding, the effect of treatment could be overestimated, whereas it could underestimate or even not be able to differentiate treatments if the time of measurement is too far from the time of feeding. An uneven distribution means that it is necessary to spend several days to have sufficient number of observations in the lacking time span, to be able to extrapolate data without associating too much uncertainty. To secure datapoints diurnally without having too large statistical variation, it is recommended to use a period of between 3 to 5 weeks in a free stall system (Hegarty, 2013; Hristov et al., 2018). Furthermore, a daily variance must be compensated for by ensuring enough data, as a

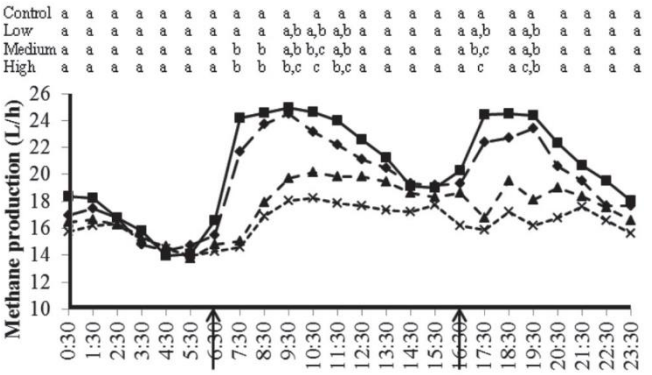

Figure 6: The CH_4 production over a 24-h period for dairy cows fed with different treatments; ■, ♦, ▲, and ✕ are treatments with 0, 5.3, 13.6 and 21.1g of NO3-/kg DM, respectively. Arrows indicate the time of feeding. Letters that are different at the same time of feeding. Letters that are different at the same time point are significantly different (P<0.05). *Source:* Olijhoek et al., 2016

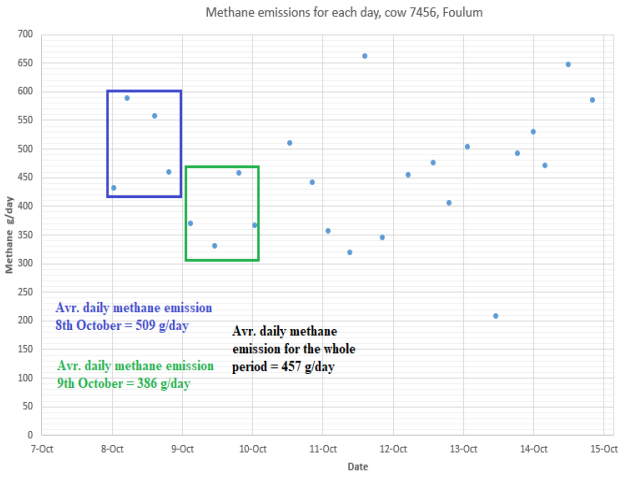

Figure 7: Daily CH_4 emissions of one cow, illustrating the importance of sufficient number of replicates and days.

Source: Exercise with Morten Maigaard, AU Foulum, 2021.

cow's CH_4 production can vary several hundred grams each day (Exercise with Morten Maigaard, AU Foulum, 2021 and illustrated in Figure 6). These temporal biases can also be affected by the diurnal rhythm. If one cow is troublesome and has a challenging time getting used to the machinery, the farmer/researcher might be prone to help. This causes the individual to be visiting the system only when the responsible person is available, which might be during the day, where the CH_4 production is high, due to the response of the feeding pattern (Crompton et al., 2011).

The response of CH_4 emission to the feeding pattern can also be a source of bias, as some cows might prefer to access the systems before eating and some after eating (Pers. communication w. Anne Louise Hellwing, AU Foulum, 2022). Thus, it is of great importance to relate the time of measurement to the time of feeding.

An additional source of behavioral bias is that a cow recalls bad experiences (Pers. communication w. Morten Maigaard, AU Foulum, 2022). For instance, if an individual experiences that the GF has run out of concentrate pellets, it is less willing to visit the system in the future. The same principle applies to a scenario, where two individuals reach for the GF at the same time, resulting in the individuals being squeezed, and thereby being less willing to visit the system again.

Technical biases include systematic errors associated with the methods. A general issue associated with the Sniffer and the GF are breath-analyzers, the CH_4 that is not excreted through eructation and breath, but instead through the hindgut fermentation (2-3%), will not be detected in the spot-samplers, but in an RC, which must be kept in mind when comparing results with the golden standard(Madsen et al., 2010).

Another systematic error, which could be an issue in the GF, would be the gas analyzer system recovery. Through personal communication with Morten Maigaard, AU Foulum, 2022, I was informed that there was a concern about a possible systematic error of the system recovery test having a deviation criterion of 8%. The recovery test is performed by injecting a known amount of CO_2 (10-30g) into the air intake, which can be compared to the measured CO_2 of the GF. This is to verify the sensors, as well as the air inflow. The concern is that 3 repetitions are made, wherefrom an average value is determined. If this mean-value does not exceed ±8% of the injected amount, the test is accepted. I was shown a scenario with very low variance, but with a consequent systematic deviation of +5% in all three tests. This could indicate a systematic error which could bias the results. A solution to this technical bias could be that an algorithm could detect if the system recovery kept systematically over-/underestimating, even though the values were lower than the deviation criterion. A related

concern is that the recovery test is made with CO_2 and not CH_4 (or other gases of interest), which makes it impossible to test for any technical bias regarding the actual measurement of CH_4. As the test is made to verify the sensors, it seems obvious to be able to verify for all gases of interest. This is mostly a theoretical problem, but if an experiment were to examine a large herd with 2 GF-units, it could be a real problem. If one of the units tend to have a consequent deviation of +5%, while the other one does not have the systematic error, the cows visiting the overestimating unit, might be recorded with a higher CH_4 production than they have in reality. As the visits are unlikely to be evenly distributed between the two units, the cows visiting the overestimating unit might be registered with less effect from an eventual treatment (Pers. communication w. Morten Maigaard, AU Foulum, 2022).

It is relevant for a CH_4 mitigating project to consider the different pitfalls of a trial setup in order to avoid or control the potential systematic errors. Thus, considerations about adaptation, herd selection, system application etc., should be made before a trial is started. These considerations will be further discussed.

4.1.4 Trial considerations and method applicability

To be able to face the challenges that may arise at farm level, the angle within the production trial must be set, including specific questions regarding the desired type of scientific outcome, which should be reflected in the type of measuring system installed.

The desired scientific outcome of a specific trait of interest is either determined on individual- or population level, which typically favors one specific experimental setup over another.

For instance, in both Brask et al., 2015 and Olijhoek et al., 2016, the target was to estimate and quantify the diurnal variation in the fermentation pattern, as the production of the flux of gasses is not constant during the circadian rhythm. In these studies, the gasses had to be accurately measured with high frequencies, in order to quantify the relationship between the treatment and the effects. Thus, it was essential for the studies to use the most precise type of measuring system, the RC.

In Lassen & Difford, 2020 examined a wide range of data was obtained, quantifying the individual traits in terms of cattle breed, ECM, bodyweight, height, DM intake and CH_4 production in a large population. This was to imply the relationship between feed efficiencies and CH_4 production to optimize breeding in a genome selection point of view. In a production trial like this, a large number of animals are required, to screen and correlate several phenotypic characteristics to a large range of

genetics, which is why the Sniffer technique was chosen, due to its high testing capacity. Lassen & Difford, 2020, emphasize the importance of a large population size in their study, but also indicate that the instrumental variance becomes less important when the number of individuals and traits is high.

Somewhat similar to Lassen & Difford, 2020, the desired outcome of the METAKS-project is to determine the CH_4 production at varying phenotypical characteristics. Conversely, the METAKS-project is to determine and quantify effects of specific treatments on the CH_4 production, rather than screening for cross-validation. Thus, one main difference between the genomic scan in Lassen & Difford, 2020 and the METAKS-project is the need to group different herds into treatment blocks, which thereby reduces the number of individuals in the treatments. Thus, the importance of minimizing the systemic variance is greater, which might justify the use of the GF system.

4.1.5 Considerations before installation of GreenFeeders in loose housed trial

To minimize the intervention of the farm to keep the environment as steady as possible and to maintain the credibility of the obtained data, the execution of the project relies on the practicality of the installation of the system. As discussed earlier, different pitfalls are related to the specific system, which should be avoided as much as possible.

First of all, the selection of the trial farm must meet various herd criteria, to obtain the desired scientific outcome. These criteria include the breed of cattle as well as the feed-/production-level. The objectives of the METAKS-project are to investigate the effects of 3-NOP on a wide range on various breeds. Especially results obtained with Jersey cows are interesting, as no 3-NOP-treatments has been performed in this breed. Thus, to reflect the investigation of 3-NOP, the selected farms should be representing various breeds.

To be able to measure production data (Feed intake and ECM production), the METAKS-project has focused on farms with installed AMS. Thereby the milk yield and composition of each cow is registered, providing the opportunity to quantify the effect of treatment more detailed, and on other parameters than the CH_4 production. Furthermore, the farms with installed AMS ensure a fair division of the cattle herd into smaller groups that somehow fits the recommended GF capacity (Pers. communication w. Martin Øvli Kristensen, SEGES, 2022). The capacity of the GF must be complied with, to have sufficient and evenly distributed measurements of all individuals, as discussed earlier. However, the study of Koning et al., 2020, challenged the capacity with two-week trials, indicating that it might be possible to increase the number of animals by prolonging the experiment and optimize

the trial. Ultimately, behavioral rhythm and traffic of the individual herd, is what matters to have sufficient observations distributed at all times of the day (Pers. communication w. Morten Maigaard, AU Foulum, 2022).

Some of these behavioral patterns can be manipulated to optimize the trial. An earlier described pitfall by the GF system is when the individuals lack to voluntarily visit the system, and thereby reduce the potential number of examined individuals and number of measurements (Jonker et al., 2020). Gunter & Beck, 2018, highlighted some of the issues regarding gas measurements in a GF with grazing cattle, which is not fully comparable to a loose housed system, as the automatic feeding system might be more unfamiliar and thereby interrupt the environment more. However, a lot of emphasis was put onto an adaption period, where the cattle was trained to use the machinery long before the beginning of the experiment. In this way, it was possible to accustom cows that otherwise would not have used the machinery, and to identify cows that only used it inconsistently, so that they could be removed from the experiment before it started. Jonker & Renand, 2020, describes initiatives that can be used to train cattle in a loose housed system. The training usually includes that the system is turned off, so that the system is quiet. Concentrate pellets can be dispensed with higher frequency and accompanied with a sound to cue the cows to the release of pellets.

By minimizing the farm modifications, it is more convenient to mimic the already installed stable interior and thereby keeping the environment stable and the data more credible. If an automatic concentrate feeder is already installed, the GF imitate a more ordinary situation in the barn, which could reduce the training period as well as increasing the success rate. Furthermore, it would reduce the burden of the farmer as well as the project manager, so that it would be more convenient to set up and execute the trial.

5 Data analysis in 3-NOP trial

In this project I got the opportunity to look into a production trial, where a cattle herd of 66 individuals was treated with 3-NOP, to assess the effect of measuring method. In the barn, both a GF and a Sniffer was installed, which makes it possible to compare the results of the methods. The provided data frame extends from 15th of December 2021 to the 15th of January 2022. The farm trial was performed in Assendrup by SEGES Innovation P/S and Aarhus University.

The GreenFeed Data was obtained by SEGES Innovation P/S and treated by the software of C-lock as described earlier. The provided format of data from C-lock was in gram CH_4 day^{-1} for each CKRDYRNR, at a measure start time- and date. The data analysis consequently consisted of processing the Sniffer data into the same format as the GF data, after which comparison analyses could be performed.

The Sniffer Data was obtained by Aarhus University, in Assendrup. The equipment was installed in an AMS as illustrated in Figure 1, wherefrom gas samples were made app. every 2 second. As the system was in association with an AMS, it was possible to extract and select the data where and which cows were present in the system at the same timestamp as the specific gas sample. The raw data was filtered in R, where specific thresholds of different parameters were applied.

5.1 Data filtering

First, the measured reference/background concentrations were subtracted from the observation samples. Reference measurements were made at specific timestamps every day for 3 minutes at 04, 10, 16, and 23 h, where no cows were present in the AMS. An average was calculated from the beginning of the 2nd minute until the 3rd minute. The 1st minute was discarded, as the measurement was not stable as illustrated in Figure 10 in the appendix. The gas samples from the cows were then subtracted from the mean reference value, for which the sampling start time was closer. That means if a cow was measured at time 06:00, the average reference value from time 04:01-04:02 would be subtracted, and not reference value from time 10 h.

The data was then filtered with respect to measuring duration and CO_2 concentration values. When a cow was recognized, the first minute would be discarded as these measurements were not representative. The cow had to become comfortable, and the gas left in the gas tube had to vanish, which depends on the gas flux through the tube. Measurements after 6 minutes would also be discarded, as 5 minutes should be sufficient. These 5 minutes were split up into 30-seconds intervals

and analyzed for CO_2 concentration peaks. The highest peak within the thresholds of 0.2-3% would represent the 30 second interval. Then an average of the possible 10 intervals was calculated, which would represent the 5 minutes of measurements. By letting the peaks represent the total emissions of the cow, the varying and non-detectable head positioning, as well as the non-even number of eructations should be taken into account as effectively as possible in the absence of a head position sensor (Pers. communication w. Martin Bjerring, AU Foulum, 2022). Through this way of analyzing the CH_4- and CO_2-peaks, we get some average measurements every time the cow is milked sufficiently with a certain detectable concentration of emissions.

The physiological parameters for each cow have been determined from data provided by the AMS and the Danish cattle database. A daily ECM, daily pregnancy stage, the bodyweight, the degree of parity and the days in milk were provided. The mean body weight for the period (16/12/21-15/1/22) was then determined with the mean being close to the median, which indicates that weight-fluctuations were distributed symmetrically around the mean. Therefore, a more period-specific partitioning of the weight was not necessary.

5.2 Statistical analysis

The physiological parameters were used to determine CH_4 production from the three models; equation 2 (Pedersen et al., 2008), equation 3 (Madsen et al., 2010), and the new updated version from Maria Holst Kjeldsen, equation 6:

$$eq\ (6): CO_2\ production\ (L\ day^{-1}) = 0.92 \cdot DIM + 62.2 \cdot ECM + 35.4 \cdot BW^{0.75} + b,$$

where b is -171, 11.8 and -33.7 for 1st, 2nd, and >2nd parity cows, respectively.

Thereby, four different CH_4 determining approaches were obtained: Pedersen, Madsen, Kjeldsen, and the GF. The boxplots in Figure 11 in appendix illustrate nine randomly picked cows that have been adapted to the GF for more than 21 days. They show general a pattern, where the GF determines the CH_4 production to be with median values below the other methods. All the Sniffers' methods have the same range of their interquartile, constantly being wider than that of the GF. I interpret this to be due to the sampling method, that there is less variance in the GF compared to the Sniffer. The less variance in the GF means that the GF is more precise, as the datapoints lie closer to the mean. This could be due to the experimental setup of the Sniffer but could also be due to the subsequent data management.

The following analysis is on herd-level, the effect of including cows that visited the GF for < 14 and < 21 days. 66 cows visited the GF in total, whereas 7 of these visited the GF for less than 14 days, and 16 cows visited the GF less than 21 days. Figure 8, shows that the quantification of CH_4 production on herd level was not affected by the individuals with fewer visits. This could be because of relatively representative visits by the cows that did not visit for many days, as well as a dilutional effect by the other cows that visited for many days that have a relatively higher effect on the mean and SD. Thus, the analysis indicate that it might not be important whether the animals that have visited the GF less than 14 or 21 days are sorted out in a herd analysis, when the number of observations is as high as in this trial.

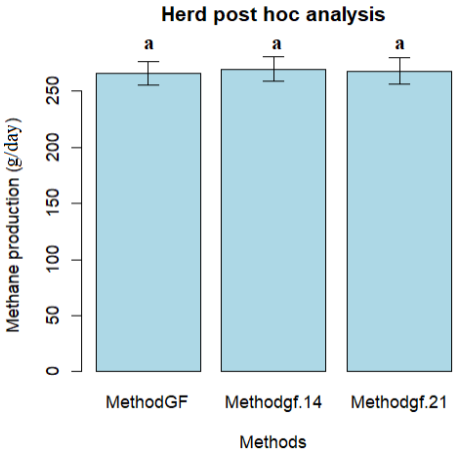

Figure 8: Post hoc analysis of the datasets including all cows (n = 66), excluding cows visiting <14 days (n = 58), and excluding cows visiting <21 (n = 49). Means were estimated to 272, 271 and 269 g/day for all cows, cows visiting <14 days and cows visiting <21 days, respectively. The std. deviations were estimated to 86, 88 and 86 for the three groups, respectively. Different letters indicate statistically significant within the 95% CI.

To compare methods on herd level, the average level of each cow, including the SD, must be determined. Figure 12 in the appendix, illustrates the relationship between the six different combinations of methods. First of all, the herd-level analysis generally confirms the individual level analysis, as Figure 12 shows the same patterns as in Figure 11. Pedersen and Madsen follow each other on a straight line, as they both determine CH_4 from the same physiological parameters. Pedersen has a higher constant, meaning that the data-points are shifted towards a higher level. Kjeldsen fit well on these two models, as it is also based on BW and ECM. When the means of the GF-cows are plotted against the Sniffers, the pattern fluctuates. Generally, the Sniffers' methods estimate CH_4 production levels higher than the levels determined by the GF. Thus, Madsen, the lowest estimating model, has the best fit to the GF. Figure 9 shows the average CH_4 production of the herd and the variance. It shows that the GF determines a general lower CH_4 level than all the Sniffers' methods. The Madsen model is closest to the GF, and then comes Pedersen, whereas the highest CH_4 production is estimated by Kjeldsen. The standard deviation is provided, which shows that the GF is more precise, due to the continuously lower standard deviation.

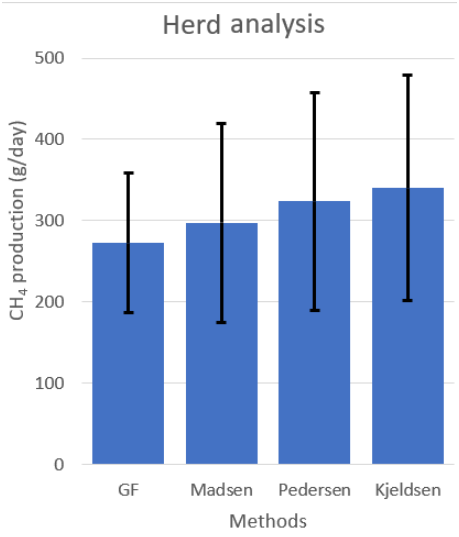

Figure 9: Herd analysis of the 4 methods on all cows (n = 66). Std. deviations were estimated to 86, 122.7, 133.4, and 138.5 for the GF, Madsen, Pedersen and Kjeldsen, respectively.

However, no statistical analysis reveals a significant difference between the methods, but since the SD of the methods overlaps so convincingly, no conclusions about the different estimations can be drawn.

5.3 Discussion

As the GF has shown a good correlation with the RC, it should be considered as the reference method in this analysis. Therefore, the results seem to show that the Sniffers' methods overestimate the CH_4 production. That statement disagrees with the findings of Maria Kjeldsen (Figure 5), where it was shown that Pedersen and Madsen generally underestimated the CH_4 production with respect to the results of RC. Thus, the GF estimates seem to fit even worse than the results of the Sniffer. A source of error could be that CO_2:CH_4 – ratio is different in the hindgut, which would affect the overall ratio in Figure 5. If the ratio is lower in the hindgut, the CH_4 production would be determined higher in the RC's than in equipment which is only collecting eructations and breath. As the hindgut fermentation accounts for only 2-3%, other sources of error must affect the results.

A competitive relationship between the GF and the Sniffer could introduce a bias between the methods. Each feed concentrates to "lure" in the cow for it to be measured. If the concentrates are of different varieties, the cows might favorize one of them, which would result in more observations for one of the methods and less for the other. It would not affect the quality of the measurements, since the software of the GF sorts out "unsuccessful" measures, and the Sniffer measures gas concentrations above a certain threshold, which ensures that the cow has been present and somehow steady in front of the measuring device. However, the type of concentrates of the systems were the same in this trial.

The effect of Bovaer could introduce a bias to the data. If the cows visit AMS/Sniffer unevenly distributed across the day, the mean of the cow would be affected by this. The cattle were generally fed at 8 am. in the morning. Thus, if the cows did not visit the Sniffer/GF in the hours after feeding, the immediate CH_4 depression effect would not be measured as significantly as it should to be representative. Figure 13 in the appendix, shows the diurnal variation of four randomly picked cows which indicate a somehow even visitation rate throughout the day in the Sniffer, whereas Figure 14 shows the visitation of the same cows in the GF. The sample I made indicates that there could be a behavioral bias in the GF, illustrated by the interval of lacking visitations. Taking cow 8186 as an example, the interval where the cow lacks visitation is in the night, a few hours before feeding. As the cows are fed Bovaer, the feeding is associated with a decrease in CH_4 production, and thus the decrease is registered, but some of the hours with high emissions are not. Therefore, this cow might

be biased by an incorrectly high production of CH_4. The opposite goes for cow 9027, where the immediate decrease in CH_4 production is not registered in the GF. As the pattern is not consistent but unclear, the bias for the individual cow might not affect the estimates on herd-level. Thus, the most reasonable source of error is the data management. I set some requirements for a measurement to be approved and made the peaks of the measurement-intervals represent the whole measuring period. As all the Sniffers' methods estimate a significantly higher level of CH_4 production, it seems like the peak-analysis is not the optimal way to analyze the interval.

As I find a difference in the quantification with either method, it is relevant to investigate whether the ranking of the cows is the same among the methods. The Sniffer is used in breeding context, where a large number of cows can be ranked and thereby it can be determined which cows are low/high emitters. As the GF is considered more precise, it should be regarded as the reference method in the ranking of low and high emitters. Table 2 illustrates the results of the ranking (full table available in Table 3 in appendix).

Table 2: Retrieval of emitting quantile from GF in the Sniffers' methods. Outline from table 3 in appendix.

Table of frequency of quartile from GF, %.	Kjeldsen			Pedersen/Madsen		
GF	Low	Medium	High	Low	Medium	High
Low	45.5	22.7	31.9	40.9	27.3	31.9
Medium	36.4	45.5	18.1	36.4	45.5	18.1
High	18.1	31.9	50	22.7	27.3	50

The ranking was done by splitting up the 66 cows of into three quantiles with 22 cows each for all methods (Madsen and Pedersen had the same ranking as they do not differ). The 22 cows with the lowest average emissions were in the 1st quartile (Low), and the 22 cows with the highest average emissions were in the 3rd quartile (High). The Low-, Medium- and High-emitting cows from the GF were identified in the other methods, and the retrieval percentage was made. The table shows that only 45.5% and 40.9% of the low emitting cows in the reference (GF) were retrieved in the Kjeldsen and Pedersen/Madsen methods, respectively, whereas only 18.1% and 22.7% of the "actual" low emitting cows were identified as high emitters by the Kjeldsen and Pedersen/Madsen methods, respectively. This could mean that the Sniffers' ability to identify low emitters is poor, which can have detrimental consequences in the selection of animals for breeding purposes.

The individual behavioral biases shown in the GF compared to the Sniffer (Figure 13 and Figure 14 in the appendix), might affect the ranking. For instance, the GF ranks Cow 9027 as a high emitter, whereas the Sniffer categorize her as a medium emitter (Table 3 in the appendix). This might be due to the aforementioned behavioral bias, where the immediate effect of Bovaer were not detected in the GF to the same degree as in the Sniffer as she had no observations just after feeding in the GF but did so in the Sniffer.

Within the Sniffers' methods, Kjeldsen and Pedersen/Madsen put emphasis on different factors in order to estimate the CO_2 production. Kjeldsen includes DIM and parity, whereas Pedersen/Madsen use pregnancy. Furthermore, the constants multiplied with ECM and BW are different, meaning that cows will be ranked differently if they express a trait to a different degree. Since ranking of the Sniffers' methods are very similar (<±1 cow per quartile), the attributive value of the different traits evidently does not differ enough to affect the outcome.

6 Conclusions

Literary conclusions.

The different properties of the methods are well studied. The test capacity of the Sniffer is higher than that of the GF, which suits it for largescale analyses, such as trait-screening for breeding purposes. Various biases are associated with spot-sampling methods, such as behavioral biases in the herd as well as technical biases, which must be taken into consideration before the preparation of a trial.

Various literary assessments have found good correlations between the GF and the golden standard, the RC, whereas the correlation between the Sniffer and the RC is worse. This lack of correlation in the sniffer is found to be associated with an underestimation by the models of the Sniffer.

Data analysis conclusions.

The data analysis on the GF-data found that there was no significant difference of the herd CH_4 production, whether cows that visited less than 14 days and 21 days were excluded or not.

The GF was found to be more precise than the Sniffer, indicated by the lower variance. The Sniffers' models seemed to overestimate the CH_4 production in the individual- and the herd-analysis compared to the results of the GF.

Since the overestimation of the Sniffer disagrees with other findings, the peak-analyzing assessment might not be the optimal way to analyze the Sniffer-data.

The ranking of the 66 cows in the GF were in substantial disagreement with the Kjeldsen- and Pedersen-/Madsen-models, since no more than 50% of the cows from the same quartiles could be retrieved. A behavioral bias could be the explanation to some of the disagreement, but since the mismatch is so significant, it can be assumed that the unequal results cannot just be explained by the bias. As one of the main functions of the sniffer is to screen for a large number of animals in order to rank them and identify the low-emitters, this finding is very interesting and could be detrimental in the breeding strategy of CH_4 reduction in agriculture.

7 Perspectives

The Sniffer is less precise than the GF due to higher variance, but the estimate of emissions should be able to be adapted, so that the mean estimates converge. The Kjeldsen-model did not converge, but rather the diverged, which also matched the international dataset illustrated in Figure 5, where the Sniffers' models generally underestimated the CH_4 production. Kjeldsen has produced a better fitted model, where the adjusted $R^2 = 0.76$, compared to the model used in this report: adjusted $R^2 = 0.68$. However, the better model requires the availability of the DMI for the individual cow, which is not realistic in an ordinary farm trial.

The lack of a head detector in the sniffer introduces a challenge, where it is difficult to obtain representative results showing both the continuous exhalation of CH_4 as well as the periodical and uneven number of eructations. Another data management approach of the Sniffer should be tested in order to inspect for more precise results.

If the entire dataset throughout all trial-periods (Control, Bovaer, Control etc.), it would have been possible to investigate the effect of Bovaer on the CH_4 measurement and thereby assess whether the share of CH_4 reduction is detected evenly throughout the methods. The large screening-capacity of the Sniffer is a valuable trait, which if it could be exploited, would be a useful tool in the near future, when the CH_4 production of livestock at specific types of farms needs to be quantified to determine the possible climate benefit of feed additives.

There was found no significant difference whether animals visiting the GF less than 14 or 21 days were excluded or not. It could be interesting to investigate the variance on herd level after only 7 days, to assess the tradeoff between the duration of the trial and the increase of variance. Thereby, it would be possible to optimize the trial by ending it whenever the variance was lowered to an acceptable level. The tradeoff is dependent on the number of cows per machine willing to visit each day, and the diurnal spread between the visits. However, the willingness to go to one machine is affected in a trial like this, where there are 2 machines, both of which "tempt" with concentrates. This means that the cow benefits less from going to one machine than if she could only get concentrate from one place. Thus, the duration of a trial like this would probably need to be increased in order to get sufficient visits from each cow.

8 Appendix

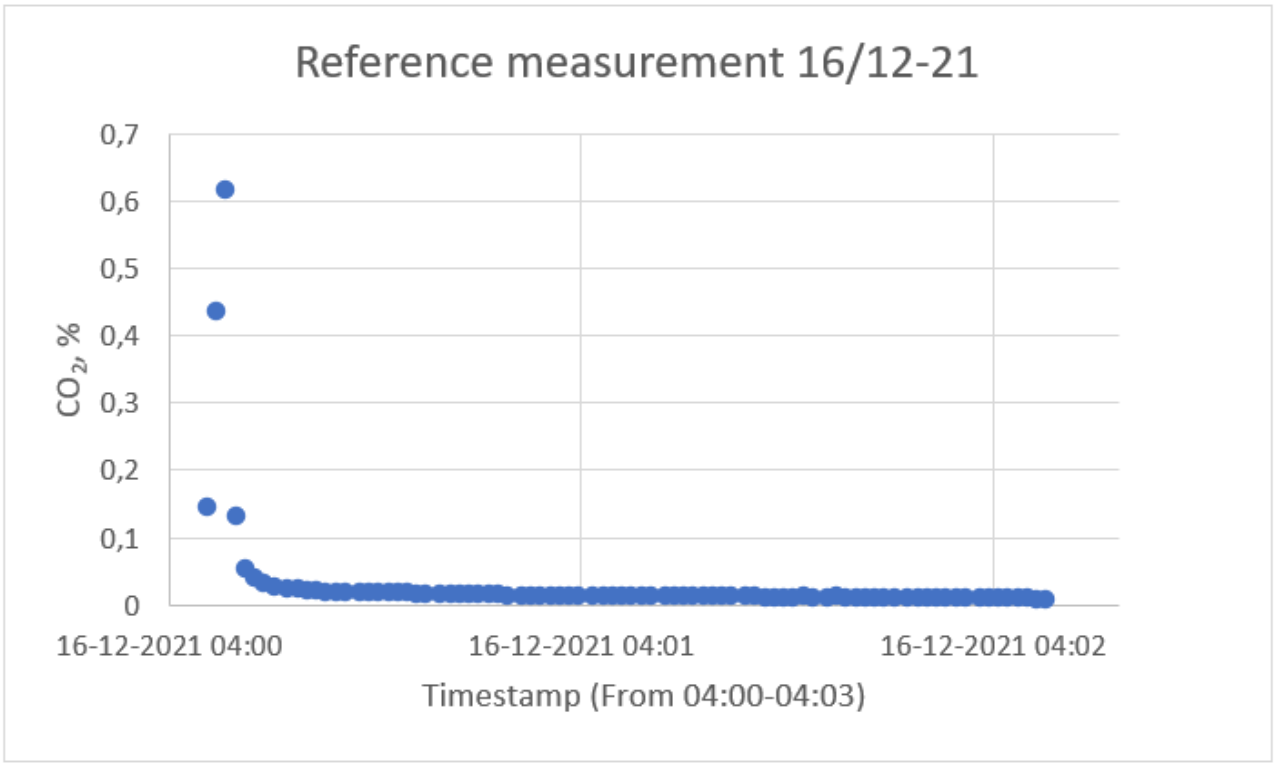

Figure 10: Reference measurements throughout approximately 3 minutes.

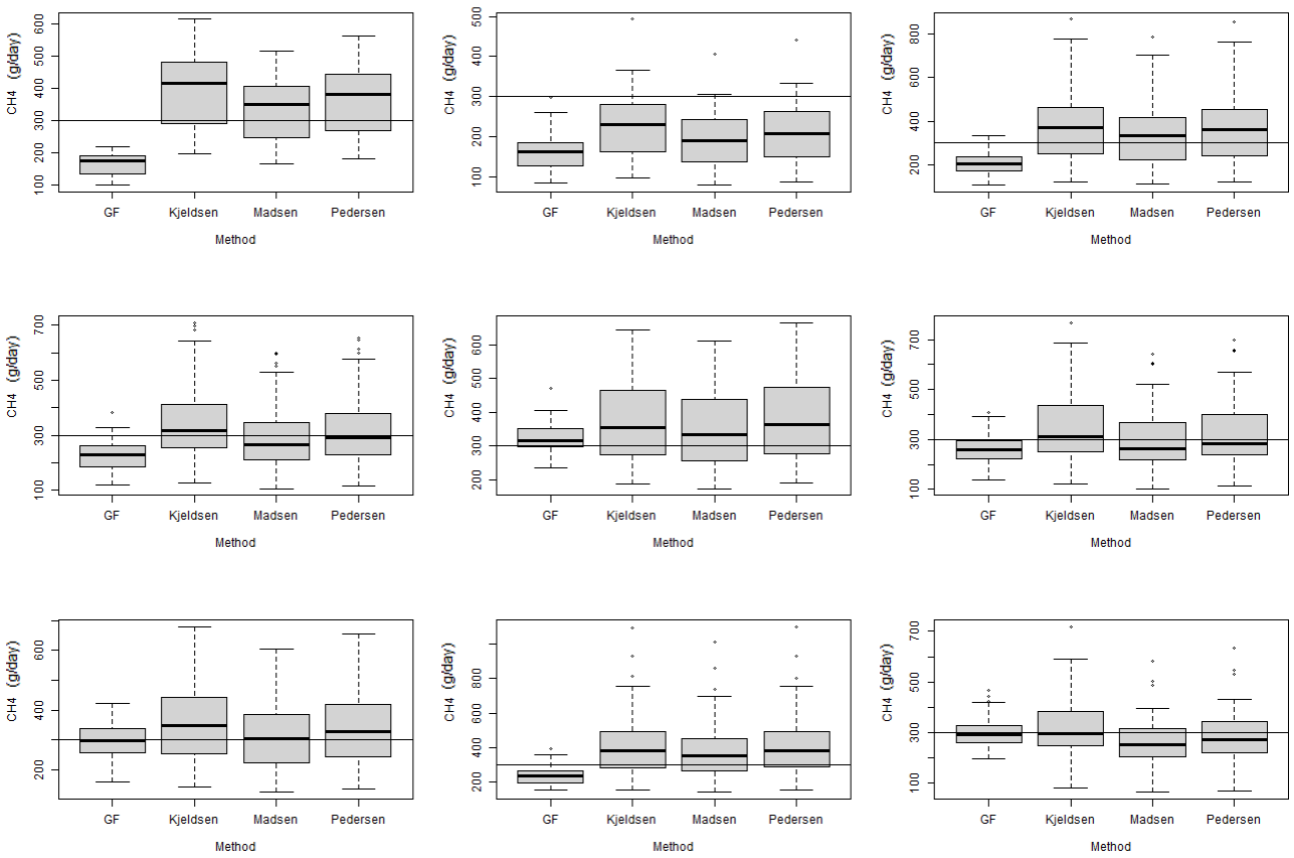

Figure 11: Boxplot of 9 randomly picked cows, that visited the GF ≥ 21 days, showing the individual CH_4-quantifying pattern of each method.

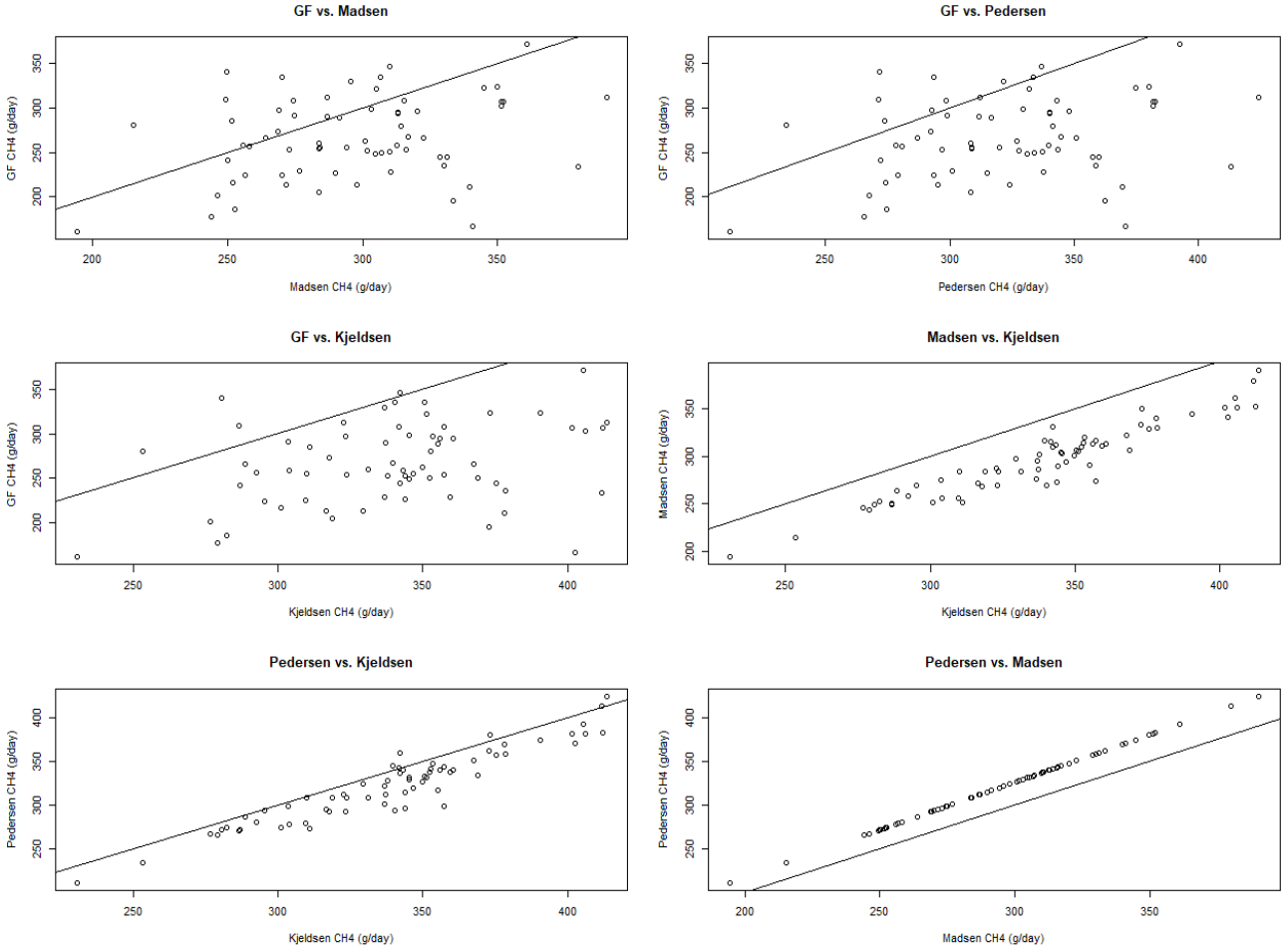

Figure 12: Mean CH_4 production values for each cow (n = 64) in all methods correlated in a scatter plot (X=Y).

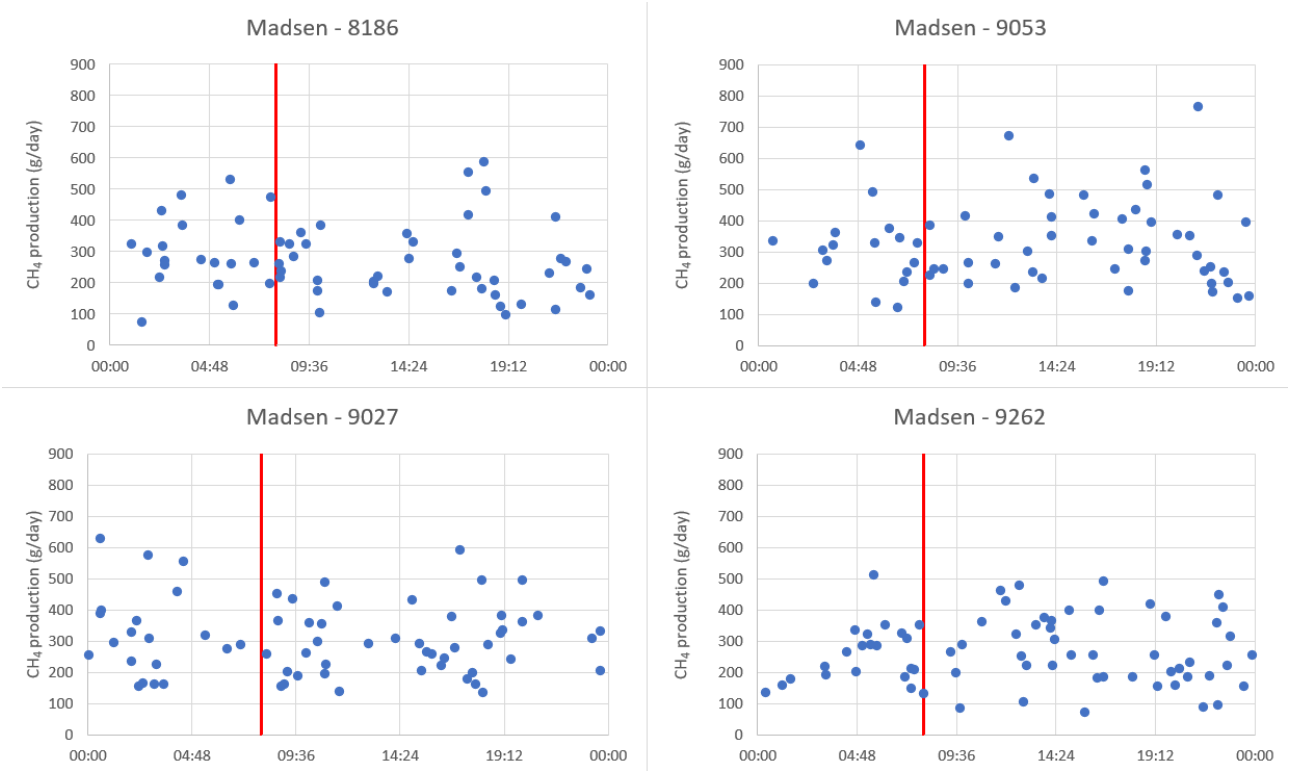

Figure 13:Diurnal variation of four randomly picked cows in the Sniffer. CH_4 quantification with relation to the Madsen model. Red line indicates time of feeding.

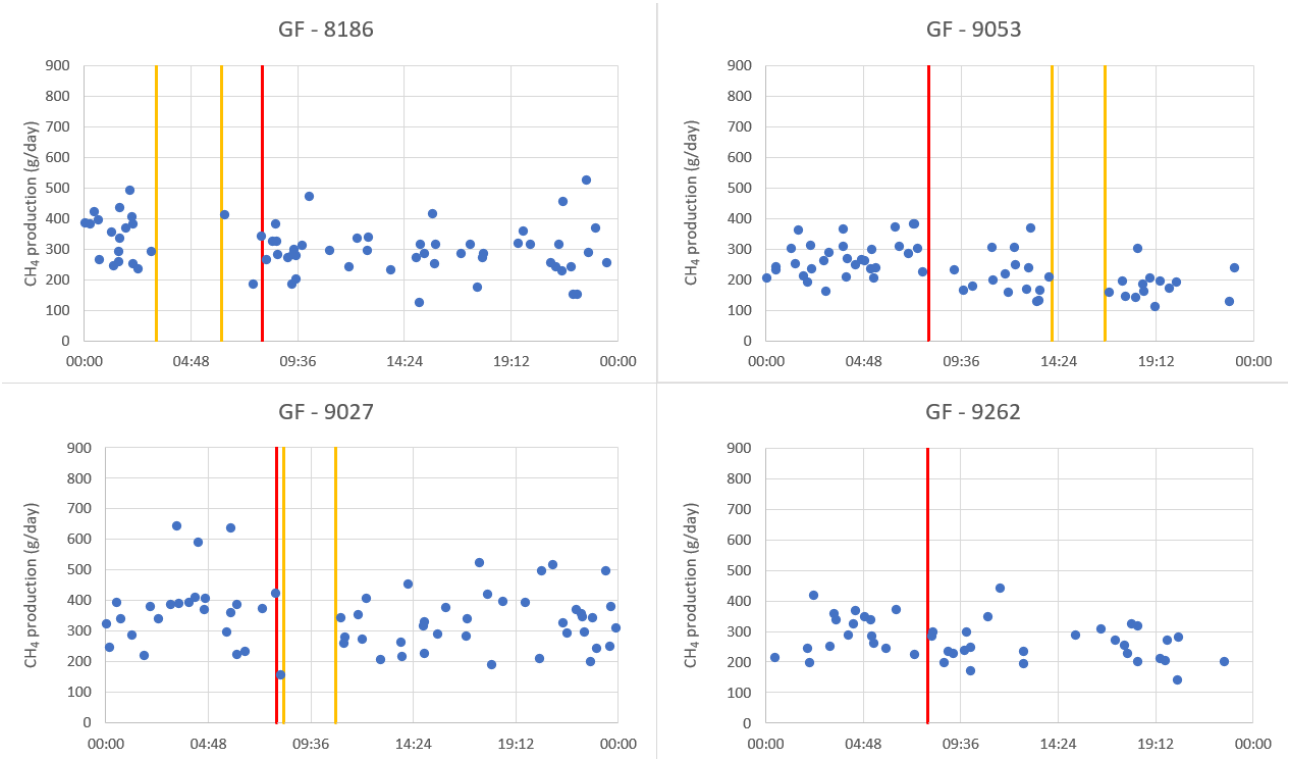

Figure 14: Diurnal variation of the GF in four randomly picked cows. Red line indicates time of feeding. The yellow lines indicate an interval that lacks visitations throughout the trial period.

Table 3:Ranking of all cows (n = 66) with relation to results in GF, showing 63.6% retrieval of low (Green) and high (Red) emitters (bottom- and top 1/3 emitters, respectively) from the GF to the Kjeldsen- and Madsen-/Pedersen-models.

ID	Mean	SD	Method		ID	Mean	SD	Method		ID	Mean	SD	Method
1314409769	161.13	44.47	GF		1314409769	231	94	Kjeldsen		1314409769	194	78	Madsen
1314409740	166.10	35.42	GF		1314409473	253	88	Kjeldsen		1314409473	215	76	Madsen
1314409765	177.57	53.18	GF		1314409758	277	112	Kjeldsen		1314409765	244	95	Madsen
1314409443	185.97	46.45	GF		1314409765	279	109	Kjeldsen		1314409758	246	100	Madsen
1314409421	195.28	66.72	GF		1314409281	281	71	Kjeldsen		1314409712	249	116	Madsen
1314409758	201.24	57.56	GF		1314409443	282	98	Kjeldsen		1314409281	250	64	Madsen
1314409441	204.89	30.39	GF		1314409712	287	134	Kjeldsen		1314409714	250	99	Madsen
1314409412	211.12	54.67	GF		1314409714	287	113	Kjeldsen		1314409067	252	102	Madsen
1314409495	213.14	57.93	GF		1314409496	289	102	Kjeldsen		1314409082	252	111	Madsen
1314409320	213.11	81.56	GF		1314409700	282	123	Kjeldsen		1314409443	253	88	Madsen
1314409082	216.13	69.95	GF		1314408647	295	99	Kjeldsen		1314409410	256	99	Madsen
1314408647	223.99	87.90	GF		1314409082	301	129	Kjeldsen		1314409013	257	89	Madsen
1314409013	224.84	73.72	GF		1314409732	304	110	Kjeldsen		1314409700	258	108	Madsen
1314409347	226.65	54.72	GF		1314409410	304	118	Kjeldsen		1314409496	264	93	Madsen
1314409425	228.16	69.73	GF		1314409013	310	105	Kjeldsen		1314409262	269	108	Madsen
1314408512	229.10	76.84	GF		1314409703	310	129	Kjeldsen		1314408546	269	100	Madsen
1314409005	233.73	51.49	GF		1314409067	311	127	Kjeldsen		1314409065	270	96	Madsen
1314409053	235.40	69.70	GF		1314409320	316	93	Kjeldsen		1314408647	270	91	Madsen
1314409714	241.52	57.20	GF		1314409262	318	127	Kjeldsen		1314409320	272	79	Madsen
1314409479	244.41	57.00	GF		1314409441	319	108	Kjeldsen		1314409291	273	110	Madsen
1314409411	244.43	85.90	GF		1314409448	323	106	Kjeldsen		1314408186	274	117	Madsen
1314409339	248.61	63.91	GF		1314408546	323	120	Kjeldsen		1314409732	275	99	Madsen
1314408784	250.32	116.22	GF		1314409403	324	135	Kjeldsen		1314408512	277	107	Madsen
1314409427	252.51	79.64	GF		1314409495	329	121	Kjeldsen		1314409441	284	97	Madsen
1314409291	252.55	68.44	GF		1314409349	331	121	Kjeldsen		1314409349	284	106	Madsen
1314409715	253.55	67.58	GF		1314408512	337	129	Kjeldsen		1314409403	284	116	Madsen
1314409403	254.10	88.73	GF		1314409298	337	149	Kjeldsen		1314409703	284	117	Madsen
1314409319	255.12	72.74	GF		1314409723	337	124	Kjeldsen		1314409723	287	105	Madsen
1314409703	255.19	71.33	GF		1314409427	338	119	Kjeldsen		1314409448	287	93	Madsen
1314409700	256.89	69.29	GF		1314409455	339	134	Kjeldsen		1314409347	290	117	Madsen
1314409410	258.17	69.73	GF		1314409065	340	122	Kjeldsen		1314408813	291	122	Madsen
1314409465	258.34	69.68	GF		1314409434	342	124	Kjeldsen		1314409319	294	123	Madsen
1314409349	260.44	65.50	GF		1314409479	342	142	Kjeldsen		1314409298	296	130	Madsen
1314409304	262.20	56.87	GF		1314409027	342	130	Kjeldsen		1314409495	298	110	Madsen
1314409327	266.03	73.14	GF		1314409465	343	158	Kjeldsen		1314409304	301	122	Madsen
1314409496	266.23	71.55	GF		1314409291	344	138	Kjeldsen		1314409427	302	107	Madsen
1314409455	267.74	67.09	GF		1314409347	344	139	Kjeldsen		1314409429	303	104	Madsen
1314409262	273.21	65.87	GF		1314409339	345	117	Kjeldsen		1314409339	305	104	Madsen
1314409323	280.08	83.88	GF		1314409429	345	117	Kjeldsen		1314409288	305	106	Madsen
1314409473	280.44	64.38	GF		1314409319	347	143	Kjeldsen		1314409462	307	120	Madsen
1314409067	285.35	67.52	GF		1314409304	350	142	Kjeldsen		1314409027	310	138	Madsen
1314408813	289.33	73.63	GF		1314409462	351	140	Kjeldsen		1314408784	310	119	Madsen
1314409723	290.29	140.91	GF		1314409288	351	122	Kjeldsen		1314409425	310	109	Madsen
1314409732	291.35	86.62	GF		1314408784	352	125	Kjeldsen		1314409465	313	135	Madsen
1314408834	294.30	103.76	GF		1314409323	353	146	Kjeldsen		1314409075	313	143	Madsen
1314409075	294.78	63.36	GF		1314409283	353	132	Kjeldsen		1314408834	313	121	Madsen
1314409283	296.94	61.70	GF		1314408813	355	149	Kjeldsen		1314409323	314	131	Madsen
1314408546	297.08	55.70	GF		1314408834	356	138	Kjeldsen		1314409434	315	110	Madsen
1314409429	298.55	99.92	GF		1314408186	357	150	Kjeldsen		1314409715	316	116	Madsen
1314408428	302.87	113.35	GF		1314409715	357	132	Kjeldsen		1314409455	317	126	Madsen
1314408816	306.96	78.57	GF		1314409425	359	157	Kjeldsen		1314409283	320	121	Madsen
1314408772	307.18	98.30	GF		1314409075	361	131	Kjeldsen		1314409327	323	131	Madsen
1314409434	307.96	95.34	GF		1314409327	368	148	Kjeldsen		1314409411	329	120	Madsen
1314408186	308.08	81.59	GF		1314409421	369	140	Kjeldsen		1314409053	330	135	Madsen
1314409712	309.53	67.33	GF		1314409305	373	127	Kjeldsen		1314409479	331	138	Madsen
1314408610	312.39	101.83	GF		1314409411	373	136	Kjeldsen		1314409421	333	122	Madsen
1314409448	312.39	63.82	GF		1314409412	375	163	Kjeldsen		1314409412	340	147	Madsen
1314409288	322.09	93.15	GF		1314409053	378	155	Kjeldsen		1314409740	341	107	Madsen
1314408530	323.16	74.33	GF		1314408530	378	160	Kjeldsen		1314408530	345	141	Madsen
1314409305	323.99	53.24	GF		1314408816	390	158	Kjeldsen		1314409305	350	120	Madsen
1314409298	329.51	83.53	GF		1314409740	402	127	Kjeldsen		1314408428	351	194	Madsen
1314409462	335.14	72.70	GF		1314409263	405	135	Kjeldsen		1314408816	351	138	Madsen
1314409065	335.29	84.00	GF		1314408428	406	225	Kjeldsen		1314408772	352	123	Madsen
1314409281	340.47	87.22	GF		1314409005	412	183	Kjeldsen		1314409263	361	121	Madsen
1314409027	346.99	103.34	GF		1314408772	412	145	Kjeldsen		1314409005	40	168	Madsen
1314409263	371.58	118.20	GF		1314408610	413	154	Kjeldsen		1314408610	390	146	Madsen

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