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Slutrapport

Projekttitel
Hitta nyckelparametrar för förbättrat grovfoderutnyttjande och lägre metanutsläpp i mjölkkor

Projektnummer: O-16-23-762

Projekttidsperiod: 2017-02-01-2021-12-30

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Del 1: Utförlig sammanfattning

Syftet med projektet var att undersöka om kor som har kapacitet att äta stora mängder grovfoder har en annan mikroflora i vämmen än kor med låg kapacitet, och om sammansättningen av mikroorganismerna och metanbildningen är kopplat till passagehastigheten. Projektet bestod av olika delstudier, den första studien var ett *in vivo* försök i ett redan finansierat försök där individuella kors förmåga att äta stora givor grovfoder studerades. För 26 utav dessa kor, utvalda då de hade högt respektive lågt intag av grovfoder (kg ts/kg kroppsvikt), studerade vi i detta projekt fodrets medelretentionstid (MRT) med hjälp av en krommarkörmetod. Vomprover samlades in för att analysera fermentationsmönstret i vommen samt sekvensering för att undersöka den mikrobiella sammansättningen och kvantitativ PCR för kvantifiering av specifika metanbildande mikroorganismer. Metanmätningar utfördes kontinuerligt på alla kor under hela laktationen. I den andra delstudien användes data från mjölkprovsanalys för att utvärdera möjligheten att skatta metanproduktionen med hjälp av mjölkspektra. En tredje studie utfördes även *in vitro*, där syftet var att särskilt studera några specifika metanbildande mikroorganismer (metanogener) som i tidigare studier visat ha ett samband till låg eller hög metanproduktion hos kor.

Det var ingen skillnad i kroppsvikt hos korna, men grovfoderintaget i kg ts skiljde mellan grupperna. Den mikrobiella sammansättning skiljde sig mellan grupperna, vilket skulle kunna förklaras av det högre foderintaget och den numeriskt lägre passagehastighet, dock inte signifikant.. Metanproduktionen skiljde inte, men det var en

Projekt har fått finansiering genom:
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tendens till skillnad i metan per kilo foder, där gruppen med lågt foderintag hade högre metanproduktion per kilo foder. Denna grupp hade också numeriskt lägre passagehastighet, dock inte signifikant. Kraftfoder och ensilage i denna studie hade väldigt lika fiberinnehåll, vilket kan vara en av förklaringarna till att vi inte såg några tydliga skillnader mellan låg och hög grupp. Mjölkspektra visade måttlig potential att användas som markör för metanemission (metanproduktion per kg mjölk), ytterligare studier bör fokusera på faktorer som kan förbättra prediktionsmodellen. Den koppling som vi kunde se mellan metanproduktion och metanogener *in vitro* var främst att mängden metanogener påverkade den totala vätgaskonsumtionen som i sin tur påverkade metanproduktionen.

Del 2: Rapporten (max 10 sidor)

Introduction

Increased forage proportion in relation to concentrate in the diet to dairy cows is positive for animal health and can bring economic benefits for the farmer. However, the forage intake capacity differ between individual cows, partly due to individual differences in size, age, lactation stage, body weight and body condition and also due to diet composition (Patel, 2012; Allen, 2021). Other possible, but less studied contributing parameters, are plausible differences in passage rate, rumen microbial community composition and/or diversity, which in turn can impact the feed utilization. The diet and feed intake are also major factors affecting methane (CH_4) production, representing important source of anthropogenic CH_4 emissions and loss of energy for the cow (Johnson and Johnson 1995), however also with large individual variation among cows (Pinares-Patiño et al., 2013; Jami and Mizhrai 2012; Patel, 2012; Danielsson, 2016). The mechanisms causing individual variation is still unclear, in spite of tremendous research effort the last decade to find mitigation strategies. The difference between animals may be caused by differences in the microbial composition and activity in the rumen, which in turn will influence the digestion of the feed (Jami and Mizhrai 2012). Ruminants harbour a large number of microbes with the capacity to convert fiber rich feeds while forming products such as volatile fatty acids (VFA), hydrogen (H_2) and carbon dioxide (CO_2) (Hook *et al.,* 2010). CH_4 is produced further on produced by methanogens (Archaea), by reduction of CO_2 by H_2.

The dominating CH_4 producer in the rumen is genus *Methanobrevibacter* (Joblin et al., 1999; Henderson et al., 2015), with *Methanobrevibacter gottschalkii* and *Methanobrevibacter ruminantium* proposed to be the main species influencing levels of CH_4 production, with a higher production in cow with a relatively higher abundance of M. *gottschalkii* versus *M ruminantium* (Shi *et al.,* 2015; Danielsson *et al.,* 2017).The reason is believed to be related to a difference between these two species to utilize hydrogen, which will impact on both the overall fermentation in the rumen as well as the level of CH_4 production (Leahy et al., 2010). The difference in methanogenic composition and abundance seems to be caused by differences in individual cows rather than diet, but at present the underlying cause for this difference has not been revealed.

For any strategy to reduce CH_4 production on individual- or farm level, accurate measurements or estimations of CH_4 are needed. Recently several new methods have been developed, such as spot sampling, flux and lasergun, which enable measurements on large number of individual animals and at lower cost compared with more commonly used methods e.g. respiration chambers (Madsen et al. 2010; Garnsworthy et al. 2012 and Huhtanen et al. 2015). However use of these methods on commercial farms is not yet suitable, due to high cost and work effort and development of an easily measured proxy of CH_4 emissions is still essential. As most Swedish dairy cows are included in the milk recording scheme, data from routine milk analysis (mid-infrared spectra, MIRS) is of interest as a potentially powerful and low-cost tool to estimate individual cow methane production.

Overall objective and aims
The main objective of the proposed project was to investigate connections between forage intake capacity, passage rate of the digesta and CH_4 emissions in dairy cows. The specific aim was to investigate whether the rumen microbiota and CH_4 formation is linked to the feed retention time and the digestibility of roughage, e.g. if cows with a high capacity to eat large amounts of forage has a different microflora and retention time in the rumen compared to cows with low forage intake capacity. An additional aim was also to investigate the possibility to predict CH_4 production from milk spectra.

Our hypothesis was that differences in forage intake capacity results in variations in CH_4 production, and that these differences can be explained by different passage rates, feed digestion and microbial community structure, which most likely are interlinked.

Material and methods

Animals, experimental design and housing
The present project was a part of a previously published study by Karlsson et al. (2020), (project number O-15-20-337) where forage intake capacity (FIC) was studied at the Swedish University of Agricultural Sciences at Lövsta, Uppsala, Sweden. In total, 37 dairy cows (Holstein (HO) and Swedish Red breed (SR)) were included in the FIC study over one full lactation period. Cows were randomly divided into two treatment groups fed two different levels of concentrates and forage *ad libitum*. All cows were housed in a free-stall barn with an automatic milking system (DeLaval VMS™; DeLaval, Tumba, Sweden); milk yield was recorded during all milkings. Roughage intake was registered at each feeding occasion (BioControl, Rakkestad, Norway) and concentrates were individually fed in concentrate feeders evenly throughout the day. The details of experimental design, feed formulation, chemical composition, and the main findings related to milk production, energy balance, feed efficiency, and fertility can be found in Karlsson et al. (2020).

Passage rate study
Passage rate/mean retention time (MRT) study included 26 cows (11 HO and 15 SR) in mid lactation (median lactation week = 15 (range 12 to 18)), when cows had a fix concentrate allowance of 5.25 (low) and 10.5 kg DM (high) respectively. Cows were selected based on their FIC (forage dry matter intake (DMI) per kg bodyweight (BW)) the weeks in prior to the sampling week. Seven of the selected cows had a high concentrate diet (HCD), these were included as a control for conventional levels of concentrate, and 19 of the selected cows had a low concentrate diet (LCD). The passage rate study periods took place during seven days at four different sessions, in May, June, August and October in year 2017. Each cow was included in one study session only.

Milk Yield, Feed Intake and Body Weight
Milk and feed consumption data were collected during sampling periods and summarized into one average value per cow. The cows were automatically weighed

every time they passed through the sorting gate when leaving the feeding area, and body weight was recorded (AWS100, DeLaval International AB, Tumba Sweden) and summarized into one average value per cow per period.

Measurements and Sample Collection
Retention time of the feed in the total gastrointestinal tract (total mean retention time, TMRT) was measured with chromium (Cr) bound to neutral detergent fiber (**NDF**) in roughage as a marker (Udén et al., 1980). Cows were given a dose of feed containing 2 g of Cr. Faeces were then collected 20 times during 164 h. The samples were freeze dried for 72 hours and ground to pass through a 1 mm sieve and then sent for analysis to ALS Global (Luleå, Sweden). The analysis was performed by inductively coupled plasma mass spectrometry (ICP-SFMS). Cr-concentrations were then related to the sampling time and fitted to a curve with equation according to Udén and Sutton (1994) using Excel Solver, estimating TMRT. Passage rate was calculated as the inversion on TMRT (1/TMRT).

Methane released by eructation were measured using an infrared technique (Guardian Plus; Edinburgh Instruments Ltd., Livingston, UK), and eructation data (peak area and frequency) were used to calculate individual daily means for CH_4 emission as described in Garnsworthy et al. (2012). Methane concentrations in air was recorded at each milking for all 37 cows and collected between April 2017 and May 2018. On average, 2.2 readings per animal per day were used.

Rumen fluid was collected through a stomach tube (Geishauser, 1993), from all cows once in each sampling period and frozen at -20 ° C until further analysis. VFA was analyzed by HPLC, previously described by Westerholm et al. (2010). DNA was extracted from rumen fluid samples using 300 µL sample and the FastDNA® Spin kit for soil (MP Biomedicals, LLC). The V4 regions of 16S rRNA gene were amplified. The amplicon library was sequenced on an Illumina HiSeq 2500 platform. To further investigate the correlation between CH_4 production and certain group of *Methanobrevibacter* species, specific primers were developed and designed within this project to target *M. gottschalkii* primerpair Mgott_454F (GGCAGCTCTAGTGGTAGCAG)/Mgott_670R GCAGAACCGTTCCAGTCAGA) and *M. ruminantium*, primerpair¨: MrumF (AGATTCTCCGGAATGCTGG) /Mrum_R (GTATTCACCGCGCGATTGTG) quantified by real-time qPCR. The setup was the same as in Danielsson et al., 2017, with the difference in amplification temperature;but 70 °C for *M. ruminantium* and 68 °C for *M. Gottschalkii.*

Rumen evacuation study
To evaluate the marker method used in passage rate/MRT study, ruminal evacuations, which is considered a reliable method to measure passage rate (Huhtanen *et al.,* 2008; Volden & Larsen 2011), were performed on four cannulated cows. The evaluation study included complete ruminal evacuations at three occasions per cow, followed by a separate marker method study with faecal samplings, identical to the above presented marker method study. The rumen cannulated cows were in the same loose housing

system as the cows in the marker method study and were offered the low concentrate diet. The cows had a 12-day adaption period to the feeding and housing system before the sampling collection started. Passage rate from the rumen evacuations and MRT from Cr-marker method, on all cows, was further compared with ruminal passage rate of forage fiber NDF predicted by the Nordic feed evaluation system NorFor (Volden and Larsen, 2011; Åkerlind and Nielsen, 2019; FRC revision 2.06).

Milk spectra study

The milk spectra study included all 37 cows described in Karlsson et al. (2020) and the aim was to evaluate milk spectra as a proxy for CH_4 production. Milk samples were taken fortnightly from every milking during 24 h, preserved with bronopol, and analyzed within 3 days using MIR spectra (CombiScope FTIR 300 HP, Delta Instruments B. V., Drachten, the Netherlands). Each full MIR spectra data consisted of 935 variables or wavenumbers ranging from 397.307 to 4000.071 cm^{-1}. Subsequently, the mean CH_4 production (g/day) and intensity (g/kg milk yield) data were averaged into one value per two weeks to match with MIR spectra data. In total, after the pre-processing (averaged fortnightly and merged), there were 1438 records, approximately n= 38 (SD= 3.4) of records per animal, that were generated for further data analysis. Methane data were averaged over two-week periods corresponding to the collected milk samples. Methane predictions were established and expressed in CH_4production (g/day) and CH_4intensity (g/kg milk yield). Partial least squares regression was used to develop models for the CH_4 prediction.

In vitro study on specific methanogens

Methanogenic species known to be dominating in rumen, e g *Methanobrevibacter gottschalkii, M. Ruminantium, M. Olleyae, M. Thaueri, M. Smithii and M. millerae* were ordered from the culture collection DSMZ (www.dsmz.de). The obtained cultures were inoculated to medium recommended by DSMZ. This medium contains rumen fluid and/or sludge fluid and several treatments, such as repeated autoclaving and sterile filtering of the medium were applied, to sterilize the medium, but still contaminating bacteria were continuously present. Thus, as an alternative, anaerobic medium already used as a standard in the lab (Westerholm et al., 2012) were also used. This medium was more defined and could be sterilized without problem. Another complication with the set-up of this experiment was that the obtained cultures from DSMZ were very low in concentration and the cells were not possible to culture. The strains were re-ordered but also DMSZ had problems why the delivery took long time and still the cell density was low and the cells did not grow. To circumvent the problem of growing the methanogenic species in pure culture we instead set up a study with rumen fluid. Rumen fluid samples were initially collected from five cannulated cows and were analyzed for presence and abundance of *M. gottschalkii,* and *M. ruminantium*, previously shown to correlate with CH_4 production (Danielsson et al., 2017, Shi et al., 2014). Based on this initial screening we selected two cows showing the largest difference in abundance of these two methanogenic species. Rumen fluid were again collected and aliquots (50 mL) were divided in to 309 ml serum bottles during flushing with N_2. For each cow

triplicate bottles with different treatments were set up: control without addition of hydrogen, addition of H_2 corresponding to 0,2 atm, addition of H_2 corresponding to 0,8 atm. The bottles were incubated for 14 days at 37ºC without shaking and gas and liquid samples were taken at regular basis for analyses of CH_4, H_2 and VFA. CH_4 and VFA were analysed by GC and HPLC according to previously described methods (Neubeck et al., 2016; Westerholm et al., 2010). H_2 was analysed by PP1 peak performer (Neubeck et al., 2016) The abundance of the different *Methanobrevibacter* species was analyzed by qPCR analysis.

Statistical Analyses

Passage rate/MRT study

After all study periods were completed, ten cows with the lowest (L-FIC) and ten with the highest forage intake capacity (H-FIC) were selected. Both groups, L-FIC and H-FIC, included seven cows from the LCD group and three from the HCD group. Statistical analyses were performed with SAS (SAS 9.3 Institute Inc., Cary, NC, 2008). Proc MEANS were used for means, min and max values within treatment group. When analysing the differences between groups to the MIXED procedure by using the model:

$$Y_{ijkl} = \text{FICgroup}_i + \text{Treatment}_i + \text{Period}_k + (\text{FICgroup} \times \text{Period})_{ik} + (\text{Treatment} \times \text{Period})_{jk} + e_{ijkl}$$

with fixed effect of FICgroup ($_{I=}$ 2), treatment group ($_j$ = 2) and period ($_k$=4) e_{ijk} is the random error. Least square means were calculated using LSMEANS/PDIFF. All effects were declared significant at $P<0.05$. When there was no effect of interaction, interaction was removed from the model.

Principal coordinate analysis (PCoA) was performed in order to find clustering patterns among the samples. The PCoA was based on Bray Curtis distance metrics and analyzed using the PAST software (http://folk.uio.no/ohammer/past/) of clustering patterns was confirmed by a distance-based non parametric MANOVA (Bray Curtis distance, PAST software).

Milk spectra study

ChemoSpec v5.3.2 package (Hanson, 2015) in R was used for pre-processing, visualization, and variable selections. The MIR spectra data were pre-processed according to the default suggestions by the package. Calibration models were developed using Partial Least Square and Principal Component Regression (pls) v2.7-3 package (Wehrens and Mevik, 2007) in R software. The calibration models were developed with a training dataset predicting CH_4 production computed as g/day as well as g/kg milk yield. The training dataset consisted of 30 animals (9 HO, 21 SR) which comprised approximately 533 data lines. The validation of the calibration models was computed using the external dataset (test dataset) that consisted of seven cow's data (2 HO, 5 SR)

Results and discussion

In the present study, we attempted to identify the individual differences between cows with different forage intake capacity, including both effect on passage rate of the feed, digestibility, CH_4 production and further the link to the microbial composition in the rumen.

Passage rate/MRT and rumen evacuation study
The FIC value for L-FIC and H-FIC groups was 0.023 ± 0.001 and 0.028 ± 0.001 kg DMI/kg BW (least square mean ± standard error), the difference in FIC was significant ($P < 0.001$) between groups. Body weight did not differ between the FIC groups but there was a tendency of difference ($P=0.057$) in total DMI between FIC groups (Table 1) average intake for L-FIC group H-FIC group was 24.4 ± 0.93 and 27.1 ± 0.90 kg of dry matter (DM), respectively. Energy corrected milk (ECM) production or for ECM per kilo DMI there were no different. Neither any of the digestibility (organic matter or dry matter) parameters nor fermentation pattern of the VFAs in rumen were different. No difference was observed in CH_4 production, g/day, but there was a tendency ($P=0.088$) observed for higher CH_4 production per DMI, g/kg, in the L-FIC group. To further explore whether the different FIC groups were related to the microbial community structure, a PCoA was performed on the bacterial composition at operational taxonomic unit (OTU) level (figure 1). This analysis exposed two separated clusters ($P = 0.0104$). The main difference at OTU level were related to different *Prevotella spp*. Relative abundance of *Prevotella* showed a tendency ($P=0.084$, SEM 0.024) to higher relative abundance in H-FIC compared to L-FIC (36.0 ± 1.29% v.s. 33.3 ± 1.93%). Mean retention time was numerical lower (48.6 ± 2.29 h) in H-FIC group compared to L-FIC group (53.4 ± 2.39 h), but there was no statistical difference. The numerical shorter retention time in H-FIC group related to higher feed intake is in line with others findings (Huhtanen and Jakkola, 1993). Longer retention time for forage compared to concentrate has also been observed previously (Colucci *et al*., 1990). An increased forage:concentrate ratio increases rumen retention time, but this seems to be more prevalent at low feed intake levels (Colucci *et al*., 1990; Huhtanen and Jaakkola, 1993). In our study it could have been expected that increased forage intake should have increased the total fibre content of the diet, but the by-product concentrate used in this study had a NDF concentration quite similar to the forage NDF, 364 ± 9.73 and 453 ± 8.91 g/kg DM, respectively.

The four cannulated cows included in the study for validation of the Cr-method had a very narrow range of ruminal passage rate in percent per hour among cows, see figure 2, thus making the validation of the marker estimation of passage rate rather weak, Anyhow, the ranking was similar with both methods. Observed values were also compared to values predicted by the NorFor model,that is based on body weight and NDF intake. Passage rate predicted by NorFor was on average 2.26 % per hour, and observed passage rate (1/TMRT) average was 1.97 % per hour The range in forage NDF passage rate predicted by NorFor (2.01-2.54 % per hour) was lower compared to the range based on Cr-marker (1.56 - 2.64 % per h), indicating that there are individual differences that are not accounted for in the NorFor model.. Even though there were a

broader span in the observed values by Cr-method, the difference between FIC groups were still too small to be significant.

Table 1. Intake, milk production, digestibility of feed organic matter (OM) and dry matter (DM), methane (CH_4) production, rumen volatile fatty acid (VFA) concentration and no. of copies of different methanogens for cows with low or high forage intake capacity (L-FIC, H-FIC), n=number of cows.

	L-FIC (n=10)	**H-FIC (n=10)**	**SEM[1]**	***P-value,* FIC group**	***P-value,* Conc. allowance**
Dry matter intake (DMI), kg/d	24.1	27.6	1.33	0.057	0.262
Milk yield (ECM[2]), kg/d	36.4	39.4	2.76	0.304	0.025
ECM/DMI, kg/kg	1.49	1.46	0.11	0.749	0.139
Body weight (BW), kg	705.2	702.6	35.57	0.941	0.863
Forage DMI/BW, kg/kg	0.024	0.028	0.0009	0.001	<0.001
Retention time (TMRT)[4], h	53.4	48.6	3.40	0.186	0.887
OM digestibility, %	71.1	69.7	3.07	0.662	0.781
DM digestibility, %	69.9	68.5	3.11	0.652	0.741
CH_4, g/day	441	441	30.1	0.986	0.367
CH_4 /DMI,g/kg	18.3	16.2	1.17	0.088	0.076
CH_4/ECM, g/kg	11.5	11.4	1.21	0.982	0.001
VFA, total, mmol/l	101.1	98.1	7.59	0.701	0.735
Acetate, mol/100 mol VFA	67.6	67.7	0.66	0.974	0.106
Propionate mol/100 mol VFA	17.5	17.9	0.50	0.458	0.998
Butyrate, mol/100 mol VFA	11.3	10.8	0.44	0.310	0.023
No. of copies/mL					
M. ruminantium	1.6×10^5	2.8×10^5	2.0×10^5	0.302	0.062
M. gottschalkii	1.6×10^6	7.0×10^5	8.9×10^5	0.307	0.343

[1]SEM - Standard error of mean

[2]TMRT – total mean retention time

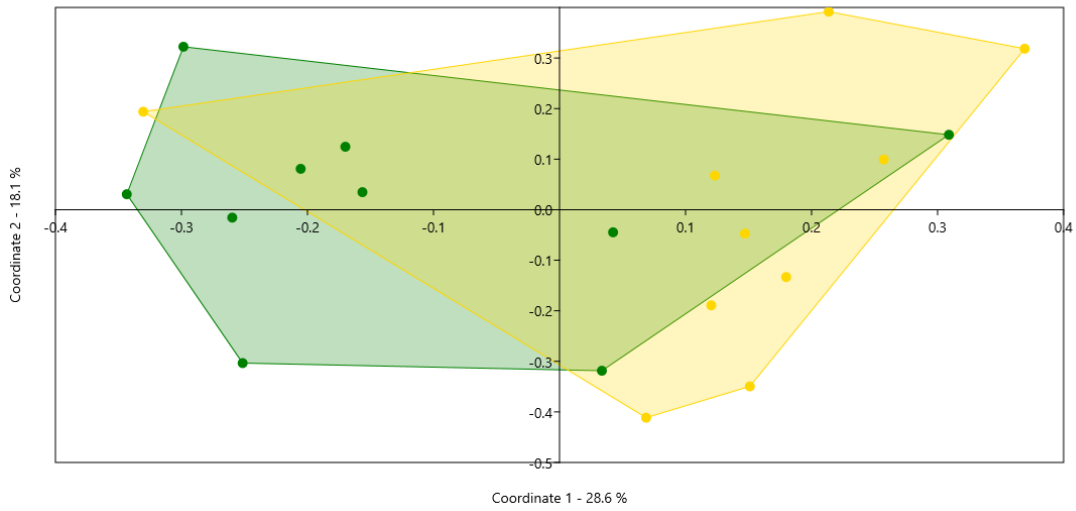

Figure 1. Principal coordinate analysis (PCoA) defining the relationship between samples based on the bacteria operational taxonomic unit (OTU) level. Colours represent different forage intake capacity (FIC), kilo dry matter intake per kilo bodyweight, groups: green= high FIC, n=10 cows, and yellow = low FIC, n=10 cows. Axis describe percentage of variance.

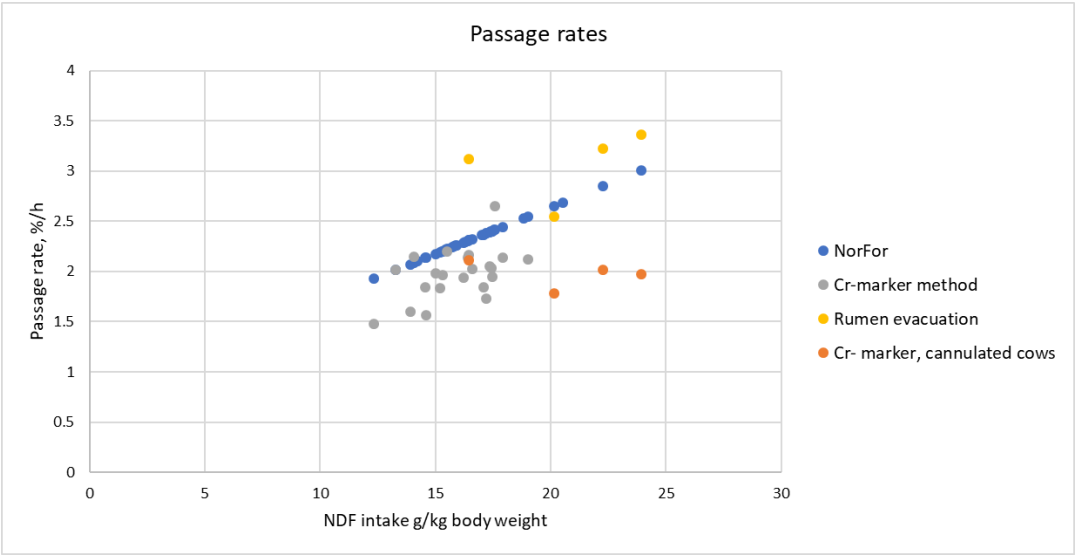

Figure 2. Comparison of observed rumen passage rate measured by the rumen evacuation method (yellow dots, kp iNDF), passage rate of indigestible NDF through total tract calculated as the inverse of TMRT (grey dots,(orange dots cannulated cows) 1/TMRT) and predicted particle passage rate of NDF in forage by NorFor model (blue dots, r_kpNDFf).

Milk spectra study

To evaluate the ability of using milk spectra as a proxy for CH_4 production, partial least squares regression was used to develop models for the prediction of CH_4. The coefficient of determination (R^2) of the validation datasets was low (0.08- 0.33) in the models with CH_4 production. Methane intensity (g CH_4 per kilo milk yield) showed a

more stable and moderate R^2 (0.26-0.34) in the validation model (table 2). Several factors might contribute to the low and moderate prediction. One of the possibilities is the variations in the measurement of CH_4 production itself. There are several different techniques in measuring CH_4 production and the variation in accuracy among the methods is quite high. In general, the choice is between high accuracy in CH_4 production measurements from few animals, and/or CH_4 measurements in many animals, by techniques measuring at certain spots, with a higher random error compared with the respiration chamber technique (Garnsworthy et al., 2019).

Table 2. Partial Least Square results with number of optimum factors, coefficient of determination (R^2) for calibration model and validation dataset, root mean square error for calibration and prediction between predicted and actual observations of CH_4 intensity, g/kg milk yield, using different predictors.

Predictors	$R^2_{calibration}$	$R^2_{validation}$	RMSEP
MIR	0.638	0.269	3.507
MIR + Lact_stage	0.648	0.275	3.491
MIR + Lact_stage + season	0.654	0.274	3.495

MIR: 576 wavenumbers from mid-infra-red spectra; RMSEP: root mean square error of prediction; Lact_stage: Lactation stage.

In vitro study on specific methanogens qPCR analyses of rumen fluid from the two selected cows showed lower abundance of *M. gottschalkii* for cow 1 as compared to cow 2, with 5.8 x 10^3 and 3.2 x 10^4 copies/mL, respectively. It was also lower abundance of *M. ruminantium* for cow 1 compared to cow 2, 7.7 x10^3 and 1.2 x 10^4 copies/mL, respectively. Moreover, rumen fluid from cow 2 had a higher *M. Ruminantium:M.gottshalkii* ratio compared to cow 1, 2.6 v.s. 1.3. The H_2 incubation test showed a comparably higher consumption rate for rumen fluid from cow 2, particularly at the lower hydrogen level (0.2 atm). Rumen fluid from cow 2 had also a slightly higher CH_4 production, which were in concordance with the higher abundance of the analysed methanogens.

Conclusions

The hypothesis of this study was that differences in forage intake (kg/kg BW) could be explained by variations in passage rate and feed digestion which would have an impact on the microbial community structure and methane production. This hypothesis could not be confirmed as no clear relations among on the measured parameters, which could partly have been caused by the lack of variation in NDF concentration in the diets.The difference in forage intake (kg DM/kg BW) between groups gave only a numerically lower mean retention time in cows with higher forage intake and no difference was observed in for methane production, VFA patterns or abundance of methanogenic archaea. However, a difference in microbial community pattern was observed between FIC-groups, mainly related to different *Prevotella* species. Milk MIRS showed moderate potential to be used as predictor for methane emission (CH_4 per kg milk), but further studies should focus on factors to improve the prediction model

Benefit for the industry and recommendations

Today it is not possible to fully understand what parameters that cause the observed individual variation in CH_4 production. In this study, the difference between high and low forage intake capacity groups was too small in combination with a high random variation in measured parameters to get any new explanations for the individual CH_4 production. Many of the presently available prediction models for CH_4 production are based on feed intake, the main driver for CH_4 production, but which is hard to measure out on farms. Other prediction models based on more easily available data like the milk spectra evaluated in this study show moderate prediction ability. It is clearly important to be aware of the degree of explanation in any prediction model especially for selecting cows for breeding purpose on low CH_4 production. In this study new primers for specific quantification of the dominant methanogenic species *Methanobrevicabter ruminantium* and *Methanobrevibacter Gottschalkii* were developed and designed, and as these species have been found to be associated with different methane emissions on individual level, this could form a basis for future research and development regarding feed efficiency and greenhouse gas mitigation.

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Del 3: Resultatförmedling

Ange resultatförmedling av projektet, inklusive titel, referens, datum, författare/talare, och länk till presentation eller publikation om tillämpligt. Planerade publiceringar (med preliminära titlar) ska ingå i tabellen. Ytterligare rader kan läggas till i tabellen.

Vetenskapliga publiceringar	Mohamad Salleh, S, Danielsson, R, Karlsson, J, & Kronqvist, C. 2022. Predicting dairy cows' methane gas production measured with infra-red sniffer method using milk mid-infrared spectroscopy (Manuscript, to be submitted to Animal January 2022)
	Karlsson J, Danielsson R, Åkerlind M, & Holtenius K. 2021. Full-lactation performance of multiparous dairy cows with differing residual feed intake. (Submitted Plos One)
Övriga publiceringar	Total digesta mean retention time in dairy cows with different abilities to consume large quantities of roughage. Danielsson R, Eklund M, Gonda H, Karlsson J, Kronqvist C & Åkerlind, M. Nordic Feed Science Conference, 2018 (muntlig presentation och skriftlig rapport)
	Förbättrat foderutnyttjande för mindre metanutsläpp. Danielsson R, Eklund M, Karlsson J, C. Kronqvist C och Åkerlind M. Vallkonferensen 2020 (muntlig presentation och skriftlig rapport)
	S. Mohamad Salleh, R. Danielsson, C. Kronqvist and J. Karlsson. 2021. Relationship between CH4 measured with sniffer method and dry matter intake in dairy cows, 625. 72nd Annual Meeting of the European Federation of Animal Science (oral presentation and abstract in proceedings)
Muntlig kommunikation	**SLF seminarium 2018 "**Forskning och innovation för ökad konkurrenskraft och hållbarhet"
Studentarbete	**Masterarbete, SLU.** The digesta passage rate among dairy cows with different abilities to consume large quantities of roughage - *its impact on the milk production, and the reliability of the chemical fibre marker.* Maria Eklund, 2019.

	Internship. Engineering Graduate School of Agronomy, in VetAgro Sup (Clermont-Ferrand - FRANCE). Maxime Mezaillies
Övrigt	Projektet har haft en egen projektsida på HUVs hemsida. https://www.slu.se/fakulteter/vh/forskning/forskningsprojekt/not/ar-det-mojligt-att-kombinera-hog-grovfoderkonsumtion-med-laga-metanutslapp-fran-mjolkkor/