Between-cow variation in digestion and rumen fermentation variables associated with methane production

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ABSTRACT

A meta-analysis based on an individual-cow data set was conducted to investigate the effects of between-cow variation and related animal variables on predicted CH₄ emissions from dairy cows. Data were taken from 40 change-over studies consisting of a total of 637 cow/period observations. Animal production and rumen fermentation characteristics were measured for 154 diets in 40 studies; diet digestibility was measured for 135 diets in 34 studies, and ruminal digestion kinetics was measured for 56 diets in 15 studies. The experimental diets were based on grass silage, with cereal grains or by-products as energy supplements, and soybean or canola meal as protein supplements. Average forage:concentrate ratio across all diets on a dry matter basis was 59:41. Methane production was predicted from apparently fermented substrate using stoichiometric principles. Data were analyzed by mixed-model regression using diet and period within experiment as random effects, thereby allowing the effect of experiment, diet, and period to be excluded. Dry matter intake and milk yield were more repeatable experimental measures than rumen fermentation, nutrient outflow, diet digestibility, or estimated CH₄ yield. Between-cow coefficient of variation (CV) was 0.010 for stoichiometric CH₄ per mol of volatile fatty acids and 0.067 for predicted CH₄ yield (CH₄/dry matter intake). Organic matter digestibility (OMD) also displayed little between-cow variation (CV = 0.013), indicating that between-cow variation in diet digestibility and rumen fermentation pattern do not markedly contribute to between cow-variation in CH₄ yield. Digesta passage rate was much more variable (CV = 0.08) between cows than OMD or rumen fermentation pattern. Increased digesta passage rate is associ-

ated with improved energetic efficiency of microbial N synthesis, which partitions fermented substrate from volatile fatty acids and gases to microbial cells that are more reduced than fermented carbohydrates. Positive relationships were observed between CH₄ per mol of volatile fatty acids versus OMD and rumen ammonia N concentration versus OMD; and negative relationships between the efficiency of microbial N synthesis versus OMD and digesta passage rate versus OMD, suggesting that the effects of these variables on CH₄ yield were additive. It can be concluded that variations in OMD and efficiency in microbial N synthesis resulting from variations in digesta passage contribute more to between-animal variation in CH₄ emissions than rumen fermentation pattern.

Key words: digestibility, rumen fermentation, passage rate, variance component

INTRODUCTION

Enteric CH₄ emissions from ruminants represent a loss of dietary energy and contribute to greenhouse gas emissions. Depending on feeding level and diet composition, 2 to 12% of feed gross energy (GE) can be lost as CH₄ (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995). Thus, strategies that mitigate CH₄ emissions are not only environmentally beneficial, but can also result in greater efficiency of feed energy utilization by the animal. Methane production in cattle is strongly and positively correlated with DMI (e.g., Yan et al., 2000; Hristov et al., 2013). Because CH₄ can be produced only from potentially digestible substrate, CH₄ production in the rumen can be expected to be positively related to feed digestibility. In addition, diet composition, which is closely associated with digestibility, influences passage and digestion kinetics of feed particles in the gastrointestinal tract, and finally CH₄ production.

Forage-to-concentrate ratio and dietary fat content are important variables influencing CH₄ emissions per unit intake (CH₄ yield; Hristov et al., 2013; Ramin and Huhtanen, 2013). Low CH₄ yields have been reported

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when high-concentrate feedlot diets are fed to growing cattle (Johnson and Johnson, 1995), reflecting increased propionate production from the higher supply of starch in these diets. Conversely, fat supplementation clearly decreases CH₄ production (Moss et al., 2000). In addition to manipulation of diet composition, many other mitigation strategies (e.g., ionophores, electron acceptors, and plant bioactive compounds) have been extensively studied (Hristov et al., 2013).

The common assumption that CH₄ production is affected mainly by the diet has been challenged since the large variation in CH₄ emissions also has been attributed to animal factors (Ellis et al., 2007; Yan et al., 2009). In a study by Blaxter and Clapperton (1965), the coefficient of variation between-animal of CH₄ yield was 7 to 8% in a respiration chamber study with sheep and cattle fed restrictively. In dairy cows fed ad libitum, the coefficient of variation for CH₄ yield was considerably greater (8–18%) when measured by the SF₆ technique (Vlaming et al., 2008). Studies conducted in sheep have shown that the variation in ruminal digesta retention time or passage rate is related to CH₄ emissions, with high CH₄ emitters having a larger rumen volume and digesta pools than low emitters (Pinares-Patiño et al., 2003; Pinares-Patiño et al., 2011; Goopy et al., 2014). Other studies have shown that the host animal controls the archaea populations in the rumen (Weimer et al., 2010; Roehe et al., 2016). Although deep metagenomic and metatranscriptomic sequencing has shown similar abundance of methanogens and methanogenesis pathway genes in high and low CH₄ emitters, the transcription of methanogenesis pathway genes was substantially increased in sheep with high CH₄ yields (Shi et al., 2014). The mechanisms explaining the between-animal variation in CH₄ emissions are not fully understood. The examination of the between-animal differences in a large data set originating from variations in digestion physiology and rumen microbial ecology could help to elucidate it.

Because the animal variation is likely to be under genetic control, one option to mitigate CH₄ emissions that has been suggested is to select for animals that emit less. Pinares-Patiño et al. (2013) demonstrated that there is repeatable individual variation in this trait and part of this variation is genetic, but that the heritability estimate was lower for CH₄ yield than for total daily CH₄ emissions (0.13 and 0.29, respectively). Therefore, in addition to heritability, further progress in genetic selection for low CH₄ emitters also depends on better understanding of the variables involved in the observed between-animal variation of this trait. The objective of the present study was to evaluate between-cow variation and repeatability in digestion and fermentation variables contributing to CH₄ emis-

sions using a large data set from physiological digestion studies using a meta-analytical approach.

MATERIALS AND METHODS

Data

A meta-analysis was conducted to evaluate repeatability and between-animal variation in digestion and fermentation variables related to CH₄ yield in dairy cows. The data were taken from studies on rumen-cannulated dairy cows, conducted using either a Latin square or switch-back design, in the Nordic countries: Finland (30 studies, 117 diets), Sweden (8 studies, 27 diets), and Norway (2 studies, 10 diets). The complete data set consisted of 637 cow/period observations, which were considered to be the experimental unit (Supplemental data file; <https://doi.org/10.3168/jds.2016-12206>). A minimum pre-condition for inclusion of a study in the meta-analysis was that feed intake, BW, milk production data, and rumen fermentation variables were reported. In addition, diet digestibility in the total tract was determined in 34 studies, omasal flow of nutrients in 26 studies, and ruminal pool sizes and digestion kinetics in 15 studies.

The mean forage-to-concentrate ratio of the diets was 59:41 on a DM basis. The concentrate supplements consisted principally of cereal grains, fibrous by-products from the food industry, and protein supplements, typically canola and soybean meal. Grass silage was the main forage source, but in 9 studies it was partly replaced with legume or whole-crop cereal silage. The diets were fed ad libitum either as TMR (30 studies) or fixed amounts of concentrate with forage ad libitum (10 studies). In omasal flow studies, the intake was usually restricted to 95% of ad libitum intake to avoid variations in daily intake during digesta sampling. When some chemical components (starch and fat in concentrate ingredients) were not reported in a specific study ($n = 6$), the missing values were taken from Finnish feed table values (LUKE, 2016). Production measurements included BW, feed intake, diet chemical composition, milk yield, and milk composition (fat, protein, lactose, and MUN). Energy-corrected milk was calculated according to Sjaunja et al. (1991).

Diet digestibility was determined by total feces collection (27 studies) or by fecal spot sampling (7 studies) using acid-insoluble ash (Van Keulen and Young, 1977) or indigestible neutral detergent fiber (iNDF; Huhtanen et al., 1994) as internal markers. Digesta flow measurements were conducted in 26 studies using the omasal sampling technique (Ahvenjärvi et al., 2000) with the triple-marker system (France and Siddons, 1986). Microbial N synthesis was determined using ¹⁵N

as a microbial marker except for 2 studies where purine-based derivatives were used. Rumen pool size was determined by manual rumen evacuation and digestion and passage kinetic variables were calculated using the compartmental flux method (Ellis et al., 1994). The animals were fed twice daily. Rumen fluid samples were mostly collected over 12 h after morning feeding. The VFA ratios acetate/propionate and propionate/butyrate were calculated, and the lipogenic:glucogenic ratio of VFA was determined as (acetate + butyrate)/propionate. Production of both CH₄ and CO₂ per mole of VFA (**CH₄VFA**; **CO₂VFA**) was calculated based on VFA stoichiometry (Wolin, 1960):

$$\begin{aligned} \text{CH}_4\text{VFA (mmol/mol of VFA)} = \\ 0.5 \times C_2 - 0.25 \times C_3 + 0.5 \times C_4, \end{aligned} \quad [1]$$

$$\begin{aligned} \text{CO}_2\text{VFA (mmol/mol of VFA)} = \\ 0.5 \times C_2 + 0.25 \times C_3 + 1.5 \times C_4, \end{aligned} \quad [2]$$

where C₂, C₃, and C₄ are molar proportions (mmol/mol) of acetate, propionate, and butyrate, respectively, in the sum of these VFA.

Total CH₄ was calculated from OM apparently digested in the rumen (**OMADR**) and rumen fermentation stoichiometry using the following equations:

$$\begin{aligned} \text{OMADR (mol of hexose; C}_6\text{ mol/d)} = \\ [\text{OM intake (kg/d)} - \text{omasal OM flow (kg/d)}] / \\ 0.162 \text{ (kg/mol)}, \end{aligned} \quad [3]$$

where the coefficient 0.162 is the molecular weight of hexose.

The VFA production per unit of hexose (C₆) fermented was calculated using stoichiometric principles as described by Van Soest (1994):

$$\begin{aligned} \text{VFAC}_6 \text{ (mol/mol)} = 6 / (2 \times C_2 + 3 \times C_3 \\ + 4 \times C_4 + \text{CH}_4\text{VFA} + \text{CO}_2\text{VFA}), \end{aligned} \quad [4]$$

where 6 is the number of C atoms in hexose; C₂, C₃, and C₄ are molar proportions (mol/mol) of acetate, propionate, and butyrate, respectively; and CH₄VFA and CO₂VFA are CH₄ and CO₂ production per mole of VFA, respectively.

To account for the more reduced state of microbial cells (Hungate, 1966; Czerkawski, 1986; Van Soest, 1994), CH₄ emissions were adjusted to a hydrogen ion (H⁺) uptake of 8.1 g per kg cells (Czerkawski, 1986). Microbial OM flow (**MOM**) at the omasum was calcu-

lated assuming that N concentration in microbial OM was 80 g/kg. Methane production was also adjusted for biohydrogenation (BH; H-atoms) of fatty acids in the rumen, using an equation presented by Huhtanen et al. (2015b). This equation assumes a proportion of 0.95 for rate of lipolysis and 0.90 for rate of biohydrogenation. A factor of 1.8 for the average number of double bonds was considered and a molecular weight of 280 g/mol was assumed for fatty acids. Total CH₄ production was calculated as

$$\begin{aligned} \text{Total CH}_4 \text{ (g/d)} = 16 \times [(\text{OMADR (mol C}_6\text{/d)} \\ \times \text{VFAC}_6 \text{ (mol/mol)} \times \text{CH}_4\text{VFA (mol/mol)} \\ - \text{MOM (kg/d)} \times 8.1 \text{ (g/kg)}) / (4 - \text{BH}/4), \end{aligned} \quad [5]$$

where OMADR, VFAC₆, CH₄VFA, MOM, and BH are as defined above, 16 is the molecular weight of CH₄, and 4 is number of H atoms in 1 mol CH₄. No adjustments were made for CH₄ production in the hindgut.

The values of CH₄ production estimated from the amount of OMADR using stoichiometric principles were compared with values obtained using empirical equations from Yan et al. (2000) and Ramin and Huhtanen (2013). The Yan et al. (2000) equation predicts the proportion of CH₄ energy in GE from feeding level and GE intake. The CH₄ production by the Yan model was calculated as follows:

$$\begin{aligned} \text{CH}_4\text{-Yan (g/d)} = 1,000 \text{ (g/kg)} \times [-0.0078 \\ \times (\text{FL} - 1) + 0.0877] \times \text{DMI (kg/d)} \\ \times 18.5 \text{ (MJ/kg DM)} / 55.5 \text{ (MJ/kg)}, \end{aligned} \quad [6]$$

where FL is feeding level multiple of maintenance, 18.5 is assumed GE concentration (MJ/kg of DM), and 55.5 is GE of CH₄ (MJ/kg).

The original equation proposed by Ramin and Huhtanen (2013, Equation [17]) was expressed in liters per day by multiplying the model coefficients by CH₄ molecular weight of 16 g/mol and dividing it by 22.4 L/mol (conversion factor), as follows:

$$\begin{aligned} \text{CH}_4\text{-RH (g/d)} = 1,000 \text{ (g/kg)} \\ \times (3.6 - 0.25 \times \text{DMIBW} + 0.022 \times \text{OMD}_m - 0.031 \\ \times \text{EE} + 0.013 \times \text{NDF} + 0.013 \times \text{NFC}), \end{aligned} \quad [7]$$

where DMIBW is DMI/BW (g/kg), OMD_m is OM digestibility determined at the maintenance level of feeding (g/kg), and dietary concentrations (g/kg of DM) of ether extract (EE), NDF, and NFC.

Statistical Analysis

The PROC MIXED in SAS (version 9.3, SAS Institute Inc., Cary, NC) was used to calculate the variance components of the selected variables taking into account as random factors experiment (Exp), diet within experiment [Diet(Exp)], period within experiment [Period(Exp)], and cow within experiment [Cow(Exp)]. Covariance structure was defined in the model using the TYPE = VC (variance components) option in the RANDOM statement. The standard deviation and coefficient of variation for each factor were calculated as the square root of the variance estimate and standard deviation divided by the respective mean value of each factor.

Repeatability values (Rep) for the most relevant variables associated with enteric methane production were calculated as

$$\text{Rep} = \sigma^2_{\text{Cow}} / (\sigma^2_{\text{Cow}} + \sigma^2_{\text{Residual}}), \quad [8]$$

where σ^2_{Cow} and $\sigma^2_{\text{Residual}}$ are Cow(Exp) and residual variances, respectively. Repeatability values provide an estimate of the correlation between values from consecutive samples on the same cow, on the same diet, and within the same period of the same experiment.

Empirical models were developed between the variables of interest regarding their biological value by single regression analysis within the MIXED procedure of SAS (Littell et al., 2006), using the following model:

$$Y_{ij} = B_0 + B_1 X_{1ij} + b_0 + b_1 X_{1ij} + e_{ij}, \quad [9]$$

where Y_{ij} = the expected value for the dependent variable Y observed at level of j of the independent variable X in study i ; B_0 = the overall intercept (fixed effect); b_0 = the random effect of study i on the intercept ($i = 1, \dots, 40$); B_1 is the regression coefficient of Y on X_1 of Y across all studies (fixed effects), X_{1ij} = value j of the continuous variable X_1 in study i ; b_1 = is the random effect of study i on the regression coefficient of Y on X_1 in study i ($i = 1, \dots, 40$), and e_{ij} = the residual error.

The models included 2 random statements: a random intercept and slope of X_1 with SUBJECT = Diet(Exp), and a random intercept with SUBJECT = Period(Exp), using the TYPE = VC as the covariance structure for both random statements. The method = ML (maximum likelihood) statement was used in the PROC MIXED model syntax. Only one random independent variable was used to avoid overparameterized models and improve convergence (St-Pierre, 2001).

Outlier observations for predicted CH_4 production were investigated for leverage and influence by the diagnostics DFFITS_{*i*} and DFBETAS_{*j*}, where $i = 1, 2,$

$\dots, 637$ and j, i denote the j th regression coefficient in the regression equation ($= 0$ or 1) estimated without observation i , where $i = 1, 2, \dots, 637$, respectively (Belsley et al., 1980). Cut-off values suggesting that an observation warranted examination were set at $|DFFITS_i| > 2\sqrt{(p/n)}$ and $|DFBETAS_{ij}| > 2\sqrt{n}$, where p = number of variables estimated in the model and n = total number of observations in the model. Dry matter intake and OMD were used as independent variables in the model when examining outliers. In total, 18 observations for predicting CH_4 emissions (Table 3) were deleted from the data set because they were identified as outliers in the model as mentioned above.

RESULTS

Data Description

Mean and ranges of diet composition and animal production data are shown in Table 1, whereas rumen fermentation, diet digestibility, and digestion kinetics parameters are presented in Table 2. Despite the large differences in diet composition, rumen pH and the proportions of major VFA did not vary greatly compared with the proportions of minor VFA. The large variation in rumen ammonia N (**RAN**) was related to differences in feed sources, especially dietary CP concentration. The mean diet digestibility was high (OMD 740 g/kg), especially NDF digestibility (**NDFD**), as expected for dairy cows diets based mainly on good quality grass silage (CV = 0.119; Table 2). The data set did not include any low digestibility diets. Digestion rate of potentially digestible NDF (**pdNDF**) displayed large variation in forage fiber composition among diets, ranging from 2.0 to 14.0%/h.

The values for predicted CH_4 production are presented in Table 3. The CH_4 VFA exhibited low variation (CV = 0.043) considering that it also included between-diet and between-period variation. The variation was much greater (CV = 0.28) for predicted total CH_4 production (g/d), double that of the CH_4 values predicted by empirical equations. The CH_4 yield ranged from 8.9 to 37 g/kg of DMI.

Variance Components

Dry matter intake has often influenced measures of ruminal digestion and CH_4 production, but in the present study did not exert any influence on the variance component Cow(Exp) when used as a covariate in the statistical analysis, and was therefore omitted. In general, variation associated with the effect of experiment (Exp) was the largest source of variation observed in the data set (not shown). In Tables 4 to 8, the variance

Table 1. Description of diet composition, feed intake, and milk yield in the data set, which was derived from 40 experiments in dairy cows

Item	n	Mean	SD	Minimum	Maximum
Intake, kg/d					
Forage DM	637	11.1	2.68	2.5	18.9
Concentrate DM	637	7.7	2.63	0.0	17.3
Total DM	637	18.9	3.35	7.4	27.5
Diet composition, g/kg of DM					
OM	637	924	39.7	885	967
CP	637	160	21.3	121	250
NDF	637	394	54.8	246	549
Starch	605	152	64.6	0.0	400
NFC ¹	589	327	62.2	109	506
Ether extract	589	42.5	14.02	14.0	106
Fatty acids	589	29.7	12.69	13.4	84.8
Milk yield and composition					
Milk, kg/d	621	26.6	6.86	5.0	44.6
ECM, kg/d	621	26.8	6.14	5.2	47.4
Fat, g/kg	621	41.2	6.05	16.5	56.9
Protein, g/kg	621	33.9	4.47	21.3	58.0
Lactose, g/kg	621	47.1	3.46	28.9	53.7
MUN, mg/dL	435	11.0	3.93	3.3	27.0
Feed efficiency					
ECM/DMI, kg/kg	621	1.39	0.235	0.40	2.02
MNE, ² g/kg	621	292	60.5	95.0	555
BW, kg	534	619	67.0	457	808

¹NFC = 1,000 – ash – CP – ether extract – NDF.

²MNE = efficiency of N utilization for milk production, defined as milk N/N intake.

components for Diet(Exp), Cow(Exp), and Residual are presented, to separate dietary and individual animal effects.

Variance component estimates for digestion variables are presented in Table 4. The variance component Diet(Exp) was the greatest source of variation. For NDFD, the variation due to Diet(Exp) was higher than that due to Cow(Exp) (CV = 0.057 and 0.023, respectively). Repeatability of NDFD was lower (0.26) than for OMD (0.37). The observed Cow(Exp) variation in OMD in the data set was rather small (CV = 0.013). The proportion of rumen digestion in total NDF digestion varied more than the total NDF, and it was also a more repeatable measurement than total NDFD (0.34). Although highly significant and moderately repeatable (0.37) quantitatively, Cow(Exp) variation in metabolic fecal OM output (fecal OM – fecal NDF; g/kg of DMI) was small (SD = 3.9 g/kg).

The variance components for rumen fermentation patterns are shown in Table 5. Both rumen pH and total VFA were considerably more repeatable (Rep = 0.46) than molar proportions of individual VFA. For the molar proportions of acetate and propionate, the variation associated with diet was about double the between-cow variation, whereas for butyrate the diet and between-cow variance components were similar.

Overall, the between-cow variation in rumen fermentation pattern (CH₄VFA) was only 0.010, and repeatability was rather low (0.11). For the total CH₄

production predicted from fermentation stoichiometry, variations associated with Diet(Exp) and Cow(Exp) were both greater in CH₄ production predicted by empirical models (Table 6). Methane yield was less variable, both between diets and between cows, than total CH₄ production. It is worth noting that Diet(Exp) variation was larger than Cow(Exp) only for CH₄VFA, and not the other approaches for predicting enteric CH₄ production.

Estimates of variance components for N metabolism variables are presented in Table 7. The Cow(Exp) variance component for RAN was of greater magnitude than the other N metabolism variables (CV = 0.149). The repeatability of RAN was similar to that of efficiency of microbial N synthesis per kg of OMTDR (Rep = 0.35 and 0.34, respectively). Microbial N flow showed the highest repeatability value among N metabolism variables (Rep = 0.51). The extent of between-cow variation in RAN also was larger than for other N metabolism variables (CV = 0.149). In both the OM and NDF pools per kilogram of BW, the contribution of the Cow(Exp) variance component to the observed variation was larger than that of the Diet(Exp) variance component (Table 8).

Empirical Models–Univariate Regressions

Stoichiometric CH₄VFA decreased ($P < 0.01$) with increasing DMI (Table 9), but it was not significantly

Table 2. Description of rumen fermentation, digestibility, and N flow at the omasum in the data set, which was derived from 40 experiments in dairy cows

Item ¹	n	Mean	SD	Minimum	Maximum
Rumen fermentation					
Rumen pH	597	6.36	0.281	5.70	7.22
Total VFA, mmol/L	636	113	17.0	71.0	162
Molar proportion, mmol/mol					
Acetate (A)	636	654	25.3	547	729
Propionate (P)	636	181	19.7	123	287
Butyrate (B)	636	125	18.7	75.2	195
Isobutyrate	636	8.3	1.62	4.4	15.0
Valerate	571	14.7	3.87	4.1	35.8
Isovalerate	563	13.2	4.61	5.4	37.3
VFA ratios					
A/P	636	3.66	0.479	2.05	5.71
(A+B)/P	636	4.36	0.555	2.33	6.79
RAN, mg/dL	617	9.34	4.804	1.50	34.0
Total-tract digestibility, g/kg					
OM	558	740	39.9	627	892
CP	558	684	51.4	519	876
NDF	542	648	77.2	413	864
MFOM, g/kg	542	100	20.0	54	172
Flow at the omasum					
OM, kg/d	397	8.21	2.14	2.10	14.0
NDF, kg/d	405	2.88	0.986	0.385	5.71
NAN, g/d	371	433	119.5	113	732
Microbial N, g/d	404	290	84.0	71	496
Microbial N efficiency					
Microbial N, g/kg of DMI	404	15.7	2.90	8.4	23.2
Microbial N, g/kg of OMTDR	380	23.6	5.24	10.7	50.7
Rumen pool size, g/kg of BW					
DM	217	18.4	3.70	9.6	30.5
OM	217	16.6	3.47	8.5	29.0
NDF	217	10.1	2.59	3.3	18.6
RNDFD, g/kg	404	608	105.3	245	878
Ruminal digestion kinetics, %/h					
pdNDF-kd	154	4.8	1.96	1.92	14
pdNDF-kp	154	1.3	0.34	0.53	2.2
iNDF-kp	154	2.6	0.62	1.34	4.6

¹RAN = rumen ammonia N; MFOM = metabolic fecal OM; OMTDR = OM truly digested in the rumen; RNDFD = rumen NDF digestibility; pdNDF-kd = digestion rate of potentially digestible NDF (pdNDF); pdNDF-kp = passage rate of pdNDF; iNDF-kp = passage rate of indigestible NDF.

Table 3. Description of predicted CH₄ production for the data set, which was derived from 40 experiments in dairy cows

Item	n	Mean	SD	Minimum	Maximum
Stoichiometric balance ¹					
CH ₄ VFA, mmol/mol	636	358	15.4	275	404
CO ₂ VFA, mmol/mol	636	583	21.0	508	659
CH ₄ per mol of hexose	636	634	27.6	507	718
Methane emissions					
Total CH ₄ , ² g/d	360	403	111.9	142	674
CH ₄ yield, ³ g/kg of DMI	360	22.6	5.16	8.9	37.2
CH ₄ -Yan, ⁴ g/d	360	391	58.6	197	509
CH ₄ -RH, ⁵ g/d	360	381	61.0	192	539

¹Predicted from VFA stoichiometry (Equations [1] and [2]).

²Total CH₄ was estimated from the amount of OM apparently digested in the rumen adjusted by H⁺ sinks (Equation [5]).

³Calculated as total CH₄/DMI.

⁴Empirical model proposed by Yan et al. (2000; Equation [6]).

⁵Empirical model proposed by Ramin and Huhtanen (2013; Equation [7]).

Table 4. Variance component estimates of digestion variables, developed using the data set derived from 40 crossover studies in dairy cows

Item ¹	Estimate	SE	$P > Z^2$	SD ³	CV ⁴
OMD, g/kg, n = 558					
Diet(Exp)	365	58.6	<0.001	19.1	0.026
Cow(Exp)	98	20.4	<0.001	9.9	0.013
Residual	170	16.6	<0.001	13.0	0.018
NDFD, g/kg, n = 542					
Diet(Exp)	1,340	215.0	<0.001	36.6	0.057
Cow(Exp)	212	54.9	<0.001	14.6	0.023
Residual	596	59.2	<0.001	24.4	0.038
RNDFD, g/kg, n = 404					
Diet(Exp)	2,324	446.9	<0.001	48.2	0.079
Cow(Exp)	740	186.9	<0.001	27.2	0.045
Residual	1,441	167.7	<0.001	38.0	0.062
MFOM, g/kg, n = 542					
Diet(Exp)	43.9	8.23	<0.001	6.62	0.066
Cow(Exp)	14.9	4.03	<0.001	3.87	0.039
Residual	44.5	4.45	<0.001	6.67	0.066

¹OMD = total-tract OM digestibility; NDFD = total-tract NDF digestibility; RNDFD = proportion of ruminal digestibility of total NDF digestibility; MFOM = metabolic fecal OM. Exp = experiment; Diet(Exp) = diet within experiment; Cow(Exp) = cow within experiment.

²Probability of Z-value.

³Calculated as the square root of the variance component estimate.

⁴Calculated as SD divided by the respective mean value of the variable.

related to rumen pH. It was positively related to digestibility variables, RAN, and molar proportion of branched-chain VFA (**BCVFA**). For each kilogram increase in DMI, CH₄VFA decreased by 0.95 mmol/mol.

Digestibility of OM was negatively associated ($P < 0.01$) with DMI, and the variation in OMD was

closely related to NDFD and the proportion of ruminal digestion in total NDFD (Table 10). For the rumen evacuation data set, OMD was positively related to the digestion rate and negatively to the passage rate of pdNDF. For individual VFA, the molar proportions of acetate and butyrate were positively and the molar

Table 5. Variance component estimates of rumen fermentation variables, developed using the data set derived from 40 crossover studies in dairy cows

Item ¹	Estimate	SE	$P > Z^2$	SD ³	CV ⁴
Rumen pH, n = 597					
Diet(Exp)	0.006	0.0016	<0.001	0.0790	0.012
Cow(Exp)	0.018	0.0031	<0.001	0.1341	0.021
Residual	0.021	0.0019	<0.001	0.1437	0.023
Total VFA, mmol/L, n = 636					
Diet(Exp)	12.2	3.00	<0.001	3.50	0.031
Cow(Exp)	31.7	5.41	<0.001	5.63	0.050
Residual	37.5	3.42	<0.001	6.13	0.054
Molar proportions of VFA, mmol/mol					
Acetate, n = 636					
Diet(Exp)	222	34.6	<0.001	14.9	0.022
Cow(Exp)	55	11.9	<0.001	7.4	0.011
Residual	138	12.4	<0.001	11.8	0.017
Propionate, n = 636					
Diet(Exp)	108	21.1	<0.001	10.4	0.055
Cow(Exp)	21	9.8	0.014	4.64	0.025
Residual	185	16.3	<0.001	13.6	0.072
Butyrate, n = 636					
Diet(Exp)	62.6	11.8	<0.001	7.91	0.061
Cow(Exp)	43.4	9.38	<0.001	6.59	0.051
Residual	96.1	8.71	<0.001	9.80	0.075

¹Exp = experiment; Diet(Exp) = diet within experiment; Cow(Exp) = cow within experiment.

²Probability of Z-value.

³Calculated as the square root of the variance-component estimate.

⁴Calculated as SD divided by the respective mean value of the variable.

Table 6. Variance component estimates for enteric CH₄ production estimates, developed using the data set derived from 40 crossover studies in dairy cows

Item ¹	Estimate	SE	<i>P</i> > <i>Z</i> ²	SD ³	CV ⁴
CH ₄ VFA, mmol/mol of VFA, n = 636					
Diet(Exp)	60.5	11.85	<0.001	7.78	0.022
Cow(Exp)	12.1	5.52	0.014	3.48	0.010
Residual	104.2	9.18	<0.001	10.21	0.028
Total CH ₄ , g/d, n = 360					
Diet(Exp)	978	304.9	<0.001	31.3	0.078
Cow(Exp)	1,745	422.1	<0.001	41.8	0.104
Residual	2,446	330.3	<0.001	49.5	0.123
CH ₄ yield, g/kg, n = 360					
Diet(Exp)	0.79	0.518	0.063	0.889	0.039
Cow(Exp)	2.26	0.750	0.001	1.502	0.067
Residual	6.97	0.905	<0.001	2.640	0.117
CH ₄ -Yan, g/d, n = 360					
Diet(Exp)	129	31.7	<0.001	11.4	0.029
Cow(Exp)	741	130.2	<0.001	27.2	0.070
Residual	193	24.3	<0.001	13.9	0.035
CH ₄ -RH, g/d, n = 360					
Diet(Exp)	332	71.3	<0.001	18.2	0.048
Cow(Exp)	950	168.7	<0.001	30.8	0.081
Residual	270	35.1	<0.001	16.4	0.043

¹Exp = experiment; Diet(Exp) = diet within experiment; Cow(Exp) = cow within experiment; CH₄-Yan = Equation [5] to predict CH₄ production according to Yan et al. (2000); CH₄-RH = Equation [6] to predict CH₄ production according to Ramin and Huhtanen (2013).

²Probability of Z-value.

³Calculated as the square root of the variance-component estimate.

⁴Calculated as SD divided by the respective mean value of the variable.

Table 7. Variance component estimates of variables related to N metabolism, developed using the data set derived from 40 crossover studies in dairy cows

Item ¹	Estimate	SE	<i>P</i> > <i>Z</i> ²	SD ³	CV ⁴
RAN, mg/dL, n = 617					
Diet(Exp)	2.91	0.523	<0.001	1.705	0.183
Cow(Exp)	1.94	0.376	<0.001	1.391	0.149
Residual	3.53	0.321	<0.001	1.878	0.201
BCVFA, mmol/mol, n = 531					
Diet(Exp)	2.62	0.569	<0.001	1.618	0.123
Cow(Exp)	2.35	0.536	<0.001	1.533	0.116
Residual	5.17	0.503	<0.001	2.273	0.173
Microbial N, g/d, n = 404					
Diet(Exp)	412	109.7	<0.001	20.3	0.070
Cow(Exp)	952	192.8	<0.001	30.9	0.106
Residual	918	105.9	<0.001	30.3	0.104
Microbial N, g/kg of DMI, n = 404					
Diet(Exp)	0.75	0.206	<0.001	0.863	0.055
Cow(Exp)	0.37	0.153	0.008	0.606	0.039
Residual	1.85	0.213	<0.001	1.361	0.087
Microbial N, g/kg of OMTDR, n = 380					
Diet(Exp)	1.34	0.542	0.007	1.156	0.049
Cow(Exp)	3.36	0.850	<0.001	1.832	0.078
Residual	6.46	0.751	<0.001	2.541	0.108

¹RAN = rumen ammonia N; BCVFA = branched-chain VFA (isobutyrate + isovalerate); OMTDR = OM truly digested in the rumen. Exp = experiment; Diet(Exp) = diet within experiment; Cow(Exp) = cow within experiment.

²Probability of Z-value.

³Calculated as the square root of the variance component estimate.

⁴Calculated as SD divided by the respective mean value of the variable.

Table 8. Variance component estimates of rumen pool and digestion kinetics metabolism variables, developed using the data set derived from 40 crossover studies in dairy cows

Item ¹	Estimate	SE	$P > Z^2$	SD ³	CV ⁴
DM pool, g/kg of BW, n = 217					
Diet(Exp)	0.82	0.317	0.005	0.905	0.049
Cow(Exp)	5.82	1.329	<0.001	2.413	0.131
Residual	1.78	0.292	<0.001	1.335	0.073
NDF pool, g/kg of BW, n = 217					
Diet(Exp)	0.80	0.225	<0.001	0.895	0.088
Cow(Exp)	1.74	0.404	<0.001	1.321	0.130
Residual	0.69	0.111	<0.001	0.832	0.082
pdNDF-kd, %/h, n = 154					
Diet(Exp)	0.355	0.1184	0.001	0.5961	0.124
Cow(Exp)	0.313	0.1069	0.002	0.5595	0.116
Residual	0.313	0.0577	<0.001	0.5596	0.116
pdNDF-kp, %/h, n = 154					
Diet(Exp)	0.011	0.0062	0.041	0.1036	0.078
Cow(Exp)	0.010	0.0059	0.039	0.1020	0.077
Residual	0.043	0.0079	<0.001	0.2083	0.157
iNDF-kp, %/h, n = 154					
Diet(Exp)	0.048	0.0188	0.005	0.2202	0.086
Cow(Exp)	0.053	0.0193	0.003	0.2303	0.090
Residual	0.085	0.0153	<0.001	0.2909	0.113

¹Exp = experiment; Diet(Exp) = diet within experiment; Cow(Exp) = cow within experiment. pdNDF-kd = digestion rate of potentially digestible NDF; pdNDF-kp = passage rate of potentially digestible NDF; iNDF-kd = passage rate of indigestible NDF.

²Probability of Z-value.

³Calculated as the square root of the variance component estimate.

⁴Calculated as SD divided by the respective mean value of the variable.

proportion of propionate negatively related to OMD, whereas the effect of rumen pH was nonsignificant. A positive relationship ($P < 0.01$) was observed between OMD and RAN concentration, but OMD decreased ($P < 0.01$) with increased microbial N flow and efficiency of microbial N synthesis.

Rumen ammonia N concentration was positively ($P < 0.01$) associated with molar proportion of BCFVA and digestion rate of pdNDF, whereas RAN decreased ($P < 0.01$) with increased passage rate of iNDF. In addition, RAN was positively ($P < 0.01$) related to molar proportions of butyrate and butyrate:propionate ratio and negatively ($P < 0.01$) related to molar proportion of propionate (models not shown).

DISCUSSION

The observed variation in the data set covered the expected ranges in dietary chemical composition, feed intake, and milk production of dairy cow diets in the Nordic countries. In the present study, repeatability and between-cow variation estimated by variance components were used to identify suitable animal variables of nutrient digestion and rumen fermentation related to between-cow differences in CH₄ emissions. The effects of diet and period within study were statistically removed from the data so that differences in ruminal digestion relating to variation in CH₄ emissions of individual animals could be identified.

Table 9. Influence of animal variables on stoichiometry CH₄, estimated by univariate mixed model regression analysis (CH₄VFA = A + BX₁) of the meta-analysis data set derived from 40 crossover studies in dairy cows¹

X ₁	n	Units	A ²	SE	B	SE	P-value	Residual
DMI	637	kg/d	376	3.7	-0.95	0.190	<0.01	115
Rumen pH	597	pH	341	13.9	2.64	2.188	0.23	121
OMD	558	g/kg	315	14.4	0.06	0.019	<0.01	99
NDFD	542	g/kg	334	7.0	0.04	0.011	<0.01	103
RAN	617	mg/dL	352	1.7	0.68	0.153	<0.01	114
BCVFA	531	mmol/mol	353	3.4	0.31	0.152	0.05	125

¹OMD = OM digestibility; NDFD = NDF digestibility; RNDFD = rumen NDF digested; RAN = rumen ammonia N; BCFVA = branched-chain VFA (isobutyrate + isovalerate). A = intercept; B = regression coefficient; X₁ = variable.

²All P-values ≤ 0.01.

Predicted CH₄ and Between-Cow Variation

Mean predicted CH₄ production was in good agreement with the values predicted using empirical models developed from large data sets obtained using respiration chamber studies (Yan et al., 2000; Ramin and Huhtanen, 2013). Predicted CH₄ yield was slightly greater (22.6 vs. 21.2 g/kg of DM) than in the study by Brask et al. (2015). The difference can be partly related to the dietary greater proportion of corn silage in that study, where CH₄ production was predicted from nutrient digestion and fermentation stoichiometry. In the current data set, digesta flow from the rumen was determined by the triple-marker method using ¹⁵N as a marker for microbial protein, which can be considered state-of-the-art methodology. However, random errors in estimating OMADR cause even greater errors in estimates of CH₄ production because overestimation of OMADR results in underestimation of the contribution of microbial cells as a H₂ sink and vice versa. Without considering microbial cells as a H₂ sink (Czerkawski, 1986; Russell, 2002), CH₄ production calculated from nutrient digestion and fermentation balance had been clearly overestimated. Ramin and Huhtanen (2013) calculated that the contribution of microbial cells as a H₂ sink can account for about 20% of reduced CH₄ yield with increased feed intake. Wolin (1960) approach could be criticized because it assumes that all fermented substrates have a formula C₆H₁₂O₆. Some carbohydrates deviate from this general formula, but they are usually only a small part of ruminant diets (Wolin, 1960). Furthermore, possible deviations from C₆H₁₂O₆ formula could influence variance component of Diet(Exp) but not that of Cow(Exp).

Between-cow coefficients of variation were 0.104 and 0.067 for predicted total CH₄ production and CH₄ yield, respectively. Between-animal coefficient of variation values in CH₄ production reported in the literature vary, reflecting differences in feed intake and methodology. Blaxter and Clapperton (1965) reported coefficient of variation values ranging from 0.072 to 0.081 for CH₄ yield by sheep in closed-circuit respiration chambers. Much greater between-cow variation, up to coefficient of variation = 0.30, has been reported in studies using sniffer methods to determine CH₄ emissions (i.e., Garnsworthy et al., 2012; de Haas et al., 2013). In the data set from respiration chamber studies (n = 539) used by Yan et al. (2010), coefficient of variation was 0.17 despite large variation in cow and diet variables (BW = 379–733 kg; DMI = 7.5–25.0 kg/d; forage proportion = 18–100%). This suggests that the sniffer methods exaggerate between-animal differences in CH₄ production. Similarly, Huhtanen et al. (2015a) reported greater between-cow variation with the sniffer method than the flux method (GreenFeed system, C-LOCK Inc., Rapid City, SD), where the latter is based on spot sampling over several days.

Rumen Fermentation

Rumen fermentation pattern can be expected to be closely related to CH₄ production due to changes in the H₂ balance, such that high acetate and butyrate production enhance CH₄ production, whereas high propionate production reduces CH₄ emissions (Johnson and Johnson, 1995; Moss et al., 2000). Jonker et al. (2016) performed a study in sheep to evaluate the ef-

Table 10. Influence of animal variables on OM digestibility (OMD), estimated by univariate mixed model regression analysis (OMD = A + BX₁) of the meta-analysis data set derived from 40 crossover studies in dairy cows¹

X ₁	n	Units	A ²	SE	B	SE	P-value	Residual
DMI	637	kg/d	780	7.7	−2.21	0.384	<0.01	272
NDFD	542	g/kg	457	10.0	0.44	0.015	<0.01	110
RNDFD/NDFD	404	ratio	691	13.0	55.4	14.37	<0.01	270
pdNDF-kd	154	%/h	719	10.4	4.70	1.794	0.01	280
pdNDF-kp	154	%/h	800	9.6	−44.8	5.92	<0.01	211
Rumen pH	597	pH	756	26.9	−3.09	4.211	0.49	280
Acetate	636	mmol/mol	617	39.0	0.18	0.06	<0.01	274
Propionate	636	mmol/mol	779	11.7	−0.23	0.063	<0.01	275
BCVFA	531	mmol/mol	729	7.3	0.51	0.333	0.13	296
RAN	617	mg/dL	723	4.1	1.74	0.346	<0.01	268
Microbial N	404	g/d	787	6.5	−0.15	0.019	<0.01	245
Microbial N/kg of OMTDR	380	g/kg	798	7.5	−2.29	0.282	<0.01	257

¹NDFD = NDF digestibility; RNDFD = rumen NDF digested; pdNDF-kd = digestion rate of potentially digestible NDF (pdNDF); pdNDF-kp = passage rate of pdNDF; both acetate and propionate as molar proportions of VFA; CH₄VFA = stoichiometry CH₄; BCVFA = branched-chain VFA (isobutyrate + isovalerate); RAN = rumen ammonia N; OMTDR = OM truly digested in the rumen. A = intercept; B = regression coefficient; X₁ = variable.

²All P-values ≤ 0.01.

fects of graded substitution of alfalfa silage with corn silage or corn grain in a diet fed at a fixed DMI level (2% of BW) on rumen fermentation characteristics in vivo and in vitro. They observed a quadratic effect of both supplements on CH₄ yield, to a maximum at 50%, and thereafter it decreased more rapidly for the corn grain supplement. The ratio of (acetate + butyrate)/(propionate + valerate) and the propionate concentration alone were the best single predictors of CH₄ yield (Jonker et al., 2016).

In the present study, between-cow variation in rumen fermentation pattern was small (CV = 0.010 for stoichiometric CH₄VFA), confirming data in Schiemann et al. (1971) where the calculated coefficients of variation of CH₄ yield and CH₄VFA ratio (Wolin, 1960) were 0.082 and 0.014, respectively, for 8 cows fed at the maintenance level, and 0.090 and 0.013, respectively, for 8 cows fed at the production level. Pinares-Patiño et al. (2013) found a coefficient of variation for CH₄ yield of 0.098 in a study with 10 sheep, whereas the coefficient of variation value for molar proportions of acetate, propionate, and butyrate was 0.011, 0.047, and 0.036, respectively. Small between-animal variation in rumen fermentation pattern relative to the variation in CH₄ yield does not indicate a major direct effect of the rumen microbiome on between-animal differences in CH₄ emissions. Indeed, the contribution of between-cow CH₄VFA variation was considerably smaller than estimated using empirical equations considering feeding level (CH₄-Yan) or diet characteristics (CH₄-RH).

Ruminal microbiome data have been infrequently associated with data on ruminal fermentation and because methanogenic communities in the rumen can vary in response to diet and animal variables, these results have to be interpreted with caution. In lactating dairy cows with differing residual feed intake (**RFI**) phenotype, no meaningful differences in rumen microbiome were associated with rumen fermentation pattern in a study by Rius et al. (2012). The absence of differences in the rumen microbiome observed in the Rius et al. (2012) study is in line with the lack of differences in the major VFA observed in the present study. Differences in rumen microbiome structure between different animals within the same diet group often exceed those observed between different diet groups (Firkins and Yu, 2015). Between-animal differences are typically attributed to different animal genetics and host interactions with the rumen microbiome (Weimer et al., 2010). In the study of Roehe et al. (2016) sire progeny groups differed significantly in their CH₄ emissions. Ranking of the sire progeny groups based on CH₄ emissions or relative archaeal abundance was consistent. This suggested that archaeal abundance in ruminal digesta was under

host genetic control as previously reported by Guan et al. (2008).

In the present study, dietary variances in molar proportions of VFA and CH₄VFA were much greater than the corresponding animal variances, suggesting that rumen fermentation pattern was more strongly associated with differences in fermented substrates between the diets than with differences in rumen microbiome between the cows. It is possible that between-animal variation increases with increased proportion of concentrate. The sum of Cow(Exp) and residual variance of CH₄VFA was about 2-fold higher (163 vs. 76) for diets above, rather than below, 42% concentrate in diet DM. It was not possible to estimate between-animal variance for diets with >42% concentrate because there was not enough replication within the study. A higher between-animal coefficient of variation for total CH₄ production and CH₄ yield for cattle fed a high-concentrate diet compared with a high-forage diet (Herd et al., 2016; Roehe et al., 2016) is in line with this suggestion.

Firkins and Yu (2006) concluded that abundance of methanogens might not be a reliable indicator of methane emission from ruminants, a claim supported by results of Danielsson et al. (2012). In the study by Jonker et al. (2016), *Methanobrevibacter ruminantium* and *Methanospheera* spp. clades had weak to moderate correlations with CH₄ yield. Methanogen densities were not significantly different in rumen samples from high and low emitters, but archaeal community structure was significantly different in rumen samples associated with different CH₄ yields (Kittelman et al., 2014). Moreover, high emitters had higher relative abundance of species that are known to form significant amounts of H₂, whereas lower CH₄ yields were related to bacterial communities that produce relatively less H₂. In a study by Carberry et al. (2012), few significant correlations between relative microbial abundance and end products of rumen fermentation or digestibility were observed. The most obvious were negative and positive correlations between relative abundance of protozoa and molar proportions of propionate and butyrate in rumen VFA, respectively. However, it should be borne in mind that in these studies conclusions are often based on statistical associations rather than rigorous demonstration of cause and effect. Part of the inconsistency between CH₄ emissions and archaea abundance could be related to within-species expression of genes involved in the methanogenic pathway, as shown by Shi et al. (2014). In their study, the abundance of methanogens and methanogenesis pathway genes were similar between low and high CH₄ emitters, but transcription of these genes was substantially higher in high emitters than in low emitters.

In the present study, repeatability of CH₄VFA was also low (0.11), probably because of small overall between-cow variation in this trait. Interestingly, repeatability was higher (0.19) for 2 consecutive periods than for all data (0.11), or for the first and last period (0.09). Low repeatability of CH₄VFA was consistent with the sheep study by Robinson et al. (2010). Pinares-Patiño et al. (2013) found that the repeatability of CH₄ yield in sheep measured on consecutive days was high (0.89), but it was only 0.26 for measurements 2 wk apart, and 0.24 across years. These observations suggest that the microbial population in the rumen can alter with time (Welkie et al., 2010; Mullins et al., 2013). Greater between-cow variation and repeatability of traits such as digestibility, passage, and efficiency of microbial cell synthesis indicate that between-animal variation in CH₄ yield may be more closely related to these characteristics than the composition of the rumen microbiome. It is important to emphasize that CH₄VFA does not take into account digestibility or passage rate through the gastrointestinal tract and only reflects the VFA fermentation profile in the rumen.

Between-cow variation was smaller when predicted by empirical models (Yan et al., 2000; Ramin and Huhtanen, 2013) than when predicted from the amount of OMADR using stoichiometric principles in the present study. This is because those empirical models do not take into account the contribution of digestibility and rumen fermentation pattern in the estimated value of the Cow(Exp) variance component. The greater between-cow variation obtained with the model of Ramin and Huhtanen (2013) compared with that of Yan et al. (2000) is most likely because the former takes variation in diet digestibility and composition into account. Both empirical models are based on *in vivo* CH₄ measurements made in respiration chambers on animals fed grass silage.

Digestibility

The between-cow variation in OMD was small (SD = 10 g/kg; CV = 0.013). Mehtiö et al. (2016) reported a value of 12.3 g/kg for between-cow variation in OMD determined using acid-insoluble ash as an internal marker. Greater variability was reported by Berry et al. (2007) for grazing cows when digestibility was determined using alkanes as a marker, probably because that study used only one replicate per animal (SD represents between-cow and random variation). In grazing conditions, plant selection can also increase the between-cow variation in digestibility. Mean retention time (MRT; reciprocal of passage rate) of the digesta is probably the main contributor to between-cow variation in digestibility. Ørskov et al. (1988) reported a

strong negative (−0.80) correlation coefficient between passage rate of Cr-mordanted straw and digestibility for 22 cows. On average, OMD increased by 4.9 g/kg per 1 h increase in MRT. In the modeling study by Huhtanen et al. (2016), the corresponding response in OMD was 3.9 g/kg per 1 h increase in MRT. Predicted between-cow variation in OMD was 12.0 g/kg for 100 simulations using the variation in MRT of iNDF observed in the present study (CV = 0.085).

Overall, between-cow variation in digestibility can explain only a small part of the estimated variation in CH₄ production. Analysis of the data from respiration chamber studies (Yan et al., 2000, 2010; Ramin and Huhtanen, 2013) indicates that CH₄ yield decreases by about 10% per multiple of maintenance increase in feeding level. This effect is much greater than observed decreases of approximately 2 to 3% in OMD per multiple of maintenance (Yan et al., 2002; Huhtanen et al., 2009; i.e., reduced digestibility can only partly explain lower CH₄ yield with increased intake). However, it is possible that incremental digestibility produces more CH₄ than the diet on average. Schiemann et al. (1971) published individual data for 8 cows fed either at maintenance or production level, for which there was a strong positive relationship between diet digestibility and CH₄ yield in both cases. In both the respiration chamber study with dairy cows (Schiemann et al., 1971) and in the modeling study (Huhtanen et al., 2016), the slope between digestibility and CH₄ yield was about 3-fold the average CH₄ yield, suggesting that incremental digestion produced more CH₄ per unit digested OM than the diet on average. Stoichiometric CH₄ ratio was positively related to OMD, but quantitatively the effect was small; a ±1 standard deviation variation in OMD was associated with only a 0.34% difference in CH₄VFA. It is possible that between-cow differences in OMD result mainly from the digestion of the slowly digestible NDF fraction, which can produce more acetate and CH₄. Positive relationships of OMD and NDF to molar proportion of acetate, and negative relationships to propionate, support this suggestion.

Passage Rate

Digesta passage rate from the rumen or its reciprocal, MRT, has been related to between-animal differences in CH₄ production. In studies with sheep fed at fixed levels, CH₄ emissions were negatively related to fractional outflow rate from the rumen (Pinares-Patiño et al., 2003) and particulate MRT was longer for high emitters compared with low emitters (Pinares-Patiño et al., 2011; Goopy et al., 2014). The simulation study by Huhtanen et al. (2016) also showed a positive relationship between MRT and CH₄ production. In the

present study, the passage rate of iNDF was moderately repeatable (0.38). In a simulation study by Huhtanen et al. (2016), the coefficient of variation of predicted CH₄ yield was 0.052 for cows and 0.045 for sheep when the simulations were made using the variation in MRT of iNDF in the current data set. Although predicted variation in CH₄ emissions was smaller than in controlled respiration studies, both the present study and the simulation study by Huhtanen et al. (2016) indicate that a considerable proportion of between-animal variation can be related to digestive processes.

Earlier studies with sheep (Faichney, 1993) and cattle (Ørskov et al., 1988) found that the ranking of animals on the basis of rumen fractional passage rate was consistent among diets and feeding levels. Smuts et al. (1995) reported that rumen digesta retention time is a repeatable physiological trait (Rep = 0.45). As a consequence, selecting animals for low CH₄ emissions may result in animals with lowered digestion capacity of cell wall carbohydrates. The effects of digesta MRT on CH₄ emissions are smaller than the effect on diet digestibility on energy basis. The efficiency of microbial N synthesis is positively related to feed intake (Chen et al., 1992; Broderick et al., 2010). Increased intake would increase ruminal passage rate (i.e., NRC, 2001) and reduce microbial retention time, and thus increase microbial cell yield per unit energy fermentation by diluting maintenance expenditure (Russell et al., 1992). The relationship between passage rate and the efficiency of microbial N synthesis could be expected to be similar when the differences in passage rate are derived from differences in feeding level or from between-animal differences. With improved efficiency of microbial synthesis associated with reduced retention time, fermented carbon is repartitioned from VFA and gas production to microbial cells. In addition, because microbial cells are more reduced than dietary carbohydrates (Hungate et al., 1971; Czerkawski, 1986; Van Soest, 1994), the stoichiometric fermentation balance (Wolin, 1960) will overestimate CH₄ emissions (Van Soest, 1994; Russell, 2002). A significant cow effect on microbial N flow and efficiency of synthesis and moderate repeatability of these characteristics suggest that the traits related to the efficiency of microbial growth in the rumen contribute to between-cow variation in CH₄ emissions.

Studies using either empirical equations (Ramin and Huhtanen, 2013) or a mechanistic modeling approach (Huhtanen et al., 2015b) indicate that improved efficiency of microbial protein synthesis makes an important contribution to reduced CH₄ yield with increased DMI. De novo fatty acid synthesis would be an important sink for carbon diverted from fermentation, but also for reducing equivalents derived from fermentation (Hackmann and Firkins, 2015).

Relationships Between Variables Affecting CH₄ Production

Several traits related to CH₄ production varied significantly between the cows and most of these were moderately repeatable. However, overall effects of these traits on CH₄ production depend on their mutual relationships. Diet digestibility could be expected to be positively related to CH₄ yield, because more substrate is fermented in the rumen. Improved OMD was positively associated with the molar proportion of acetate and CH₄VFA and negatively with the molar proportion of propionate. Thus, the effects of OMD and CH₄VFA are additive. Because most of the variation in OMD was due to NDFD (cow variance for NDFD was 3-fold that for digestibility of ND solubles) and CH₄VFA ratio was positively related to dietary NDF concentration, incremental digestibility increased CH₄VFA.

Between-cow variation in digestibility can result from changes in digestion and passage kinetic parameters, changes in the rumen microbial population, or both. In the present study, passage rate of pdNDF predicted OMD better than digestion rate of pdNDF (residual variance 209 and 279, respectively). This suggests that variation in digestibility is related more to physiological traits of the animal regulating passage rate, rather than the rumen microbiome regulating digestion rate. The differences in CH₄ yield between low- and high-emitting sheep have been associated with digesta retention time (Pinares-Patiño et al., 2003, 2011; Goopy et al., 2014), with diet digestibility being significantly (Pinares-Patiño et al., 2011) or numerically (Goopy et al., 2014) lower in low-emitting sheep. Methane yield and OMD were higher in low-RFI (efficient) steers than high-RFI steers in a study by McDonnell et al. (2016). However, the differences in diet digestibility between low and high emitters could only partly explain the difference in CH₄ yield.

The effects of the rumen microbiome on diet digestibility could derive from increased potential NDFD, digestion rate of pdNDF, or both. In our in-house determinations of iNDF concentration by 12-d intraruminal *in situ* incubation, the between-cow variation was small compared with the observed between-cow variation in OMD (unpublished data), suggesting that the effects of the rumen microbiome on the potential extent of fiber digestion are small. Carberry et al. (2012) reported a negative correlation between relative abundance of *Prevotella* sp. and OMD. In the study by Rius et al. (2012), low-RFI cows had higher OMD than high-RFI cows, but rumen microbiome was not related to digestibility. Ample evidence indicates that cell wall degradation follows first-order kinetics with respect to substrate concentration (Waldo et al., 1972; Van Soest,

1973), and that the rate is limited by the characteristics of cell walls rather than the abundance or activity of the microbial population. In fact, in vitro studies have revealed that digestion rate of NDF does not decline until ruminal contents are diluted about 6-fold (Pell and Schofield, 1993; Mouriño et al., 2001).

Interestingly, despite significant cow variance and moderate repeatability of rumen pH, this trait was not significantly related to OMD or NDFD. It is possible that with dairy cow diets with mean rumen pH (6.4), between-cow variation ($SD = 0.13$) is not large enough to influence digestibility. In a rumen content exchange experiment by Weimer et al. (2010), ruminal pH and VFA profiles returned to those of the recipient cows much more quickly (within 1 d) than did bacterial community composition, indicating that the animal exerts ultimate control over its ruminal chemistry through salivary buffering and through absorption and passage of VFA.

The efficiency of microbial N synthesis, expressed as microbial N per kilogram of DMI, digested OM intake, or OM truly digested in the rumen, was negatively related to OMD or NDFD and positively to passage rate of pdNDF. This indicates that the effects of digestibility and microbial efficiency on CH_4 production are additive. Both digestibility and microbial efficiency are related to passage rate; digestibility, especially that of the plant cell wall fraction, decreases with increased passage rate and microbial efficiency improves, as less ATP is required for microbial maintenance. Methane production can be expected to decrease with increased passage rate because (1) less substrate is fermented, and (2) there is repartition of fermented carbon from VFA and gas production to microbial cells, (3) which in turn are more reduced than fermented carbohydrates. The efficiency of microbial synthesis was negatively correlated with molar proportions of BCVFA in the rumen. Branched-chain VFA are produced in the deamination of branched-chain AA and the changes in their concentrations reflect a balance between degradation and utilization for microbial cell synthesis (Russell and Sniffen, 1984; Bach et al., 2005).

CONCLUSIONS

The present analysis showed that variables related to animal physiology, such as variation in digesta retention time, can explain most of the between-animal variation in CH_4 yield. Only small variations were observed in rumen fermentation variables, especially stoichiometric CH_4 /VFA, suggesting a minor contribution of the rumen microbiome to CH_4 production. The variation in nutrient digestibility was also small and therefore did not account for much of the variation in CH_4 yield.

However, the variation in digestibility can be attributed at least partly to differences in digesta passage rate, which influence the efficiency of microbial cell synthesis in repartitioning fermented carbon between VFA, gas production, and microbial cells. If between-animal variation in CH_4 yield is related to digesta passage rate, care should be exercised when selecting low-emitting animals in breeding programs, as this could lead to reduced efficiency of cell wall digestion.

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