



Meta-analysis of relationships between enteric methane yield and milk fatty acid profile in dairy cattle

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ABSTRACT

Various studies have indicated a relationship between enteric methane (CH₄) production and milk fatty acid (FA) profiles of dairy cattle. However, the number of studies investigating such a relationship is limited and the direct relationships reported are mainly obtained by variation in CH₄ production and milk FA concentration induced by dietary lipid supplements. The aim of this study was to perform a meta-analysis to quantify relationships between CH₄ yield (per unit of feed and unit of milk) and milk FA profile in dairy cattle and to develop equations to predict CH₄ yield based on milk FA profile of cows fed a wide variety of diets. Data from 8 experiments encompassing 30 different dietary treatments and 146 observations were included. Yield of CH₄ measured in these experiments was 21.5 ± 2.46 g/kg of dry matter intake (DMI) and 13.9 ± 2.30 g/kg of fat- and protein-corrected milk (FPCM). Correlation coefficients were chosen as effect size of the relationship between CH₄ yield and individual milk FA concentration (g/100 g of FA). Average true correlation coefficients were estimated by a random-effects model. Milk FA concentrations of C6:0, C8:0, C10:0, C16:0, and C16:0-*iso* were significantly or tended to be positively related to CH₄ yield per unit of feed. Concentrations of *trans*-6+7+8+9 C18:1, *trans*-10+11 C18:1, *cis*-11 C18:1, *cis*-12 C18:1, *cis*-13 C18:1, *trans*-16+*cis*-14 C18:1, and *cis*-9,12 C18:2 in milk fat were significantly or tended to be negatively related to CH₄ yield per unit of feed. Milk FA concentrations of C10:0, C12:0, C14:0-*iso*, C14:0, *cis*-9 C14:1, C15:0, and C16:0 were significantly or tended to be positively related to CH₄ yield per unit of milk. Concentrations of C4:0, C18:0, *trans*-10+11 C18:1, *cis*-9 C18:1, *cis*-11 C18:1, and *cis*-9,12 C18:2 in milk fat were significantly or tended to be negatively related to CH₄ yield per unit of milk. Mixed

model multiple regression and a stepwise selection procedure of milk FA based on the Bayesian information criterion to predict CH₄ yield with milk FA as input (g/100 g of FA) resulted in the following prediction equations: CH₄ (g/kg of DMI) = 23.39 + 9.74 × C16:0-*iso* - 1.06 × *trans*-10+11 C18:1 - 1.75 × *cis*-9,12 C18:2 (R² = 0.54), and CH₄ (g/kg of FPCM) = 21.13 - 1.38 × C4:0 + 8.53 × C16:0-*iso* - 0.22 × *cis*-9 C18:1 - 0.59 × *trans*-10+11 C18:1 (R² = 0.47). This indicated that milk FA profile has a moderate potential for predicting CH₄ yield per unit of feed and a slightly lower potential for predicting CH₄ yield per unit of milk.

Key words: methane, milk fatty acid profile, meta-analysis, dairy cattle

INTRODUCTION

Enteric fermentation is the main source of greenhouse gas (GHG) emissions from dairy cattle, with enteric CH₄ amounting to 1.1 Gt per year, representing 46% of the total GHG emissions in dairy supply chains (Gerber et al., 2013). Enteric CH₄ production is among the main targets of GHG mitigation practices for the dairy industry (Hristov et al., 2013). In view of these emissions and various mitigation options, there is a clear need for simple and inexpensive measurement techniques to estimate CH₄ emissions from dairy cattle in commercial practice.

Various methods have been used to estimate CH₄ production from ruminants. Production of CH₄ can be measured in respiration chambers, which is an accurate but expensive technique, unsuitable for application on a large scale. Other methods, including the SF₆ marker and CH₄:CO₂ ratio techniques, enable CH₄ emissions to be determined in a larger number of animals but with higher between- and within-animal variation (Storm et al., 2012). For inventory and prediction purposes, many empirical equations have been developed to relate CH₄ production per day or yield per unit of feed to feed intake and composition. Such equations have major limitations in predicting effects of mitigation strategies

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at the whole-farm level (Ellis et al., 2010). Mechanistic models describing the mechanism of enteric feed degradation provide more accurate predictions of CH₄ production than do empirical models (Alemu et al., 2011). However, mechanistic models are more complex and require inputs that may not be commonly measured. In addition, forcing functions used and bias in parameter values adopted may limit the meticulousness of predicted model output. For these reasons, a simple and robust prediction equation of enteric CH₄ yield from dairy cattle based on characteristics of feed or milk would be valuable for application on a large scale in GHG mitigation practices for the dairy industry.

Milk samples are frequently used in dairy farms to assess the nutritional and health status of dairy cattle and to obtain information on losses to the environment. For example, milk urea content is used to assess protein status of the animal and to estimate N excretion (Spek et al., 2013). Several studies have related diet composition to both milk FA composition and enteric CH₄ production (e.g., Chilliard et al., 2009). Such relationships may be a result of lipid supplementation, which changes both CH₄ production and milk FA profile, or may be a result of changes in diet composition, in view of CH₄ production being associated with the VFA profile produced in the rumen (Ellis et al., 2008) and VFA in turn being precursors of milk FA synthesized de novo (Bernard et al., 2008). The odd- and branched-chain fatty acid (OBCFA) content of milk has also been shown to be related to rumen function (Vlaeminck et al., 2006a).

Chilliard et al. (2009) supplemented cattle diets with different physical forms of linseed (crude, extruded, and oil). The most positive correlations between CH₄ production (g/d) and milk FA concentrations were obtained for saturated FA (C6:0 to C16:0) and the most negative correlations for various *trans*-C18 FA. Milk OBCFA concentrations exhibited less strong correlations with CH₄ production. These relationships may only apply to linseed supplemented diets. Mohammed et al. (2011), using only dietary oilseed supplementation (sunflower seed, linseed, and canola seed), best predicted CH₄ production (g/d) by milk FA concentration of C16:0-*iso* (positive relationship) and *cis*-9 C17:1 (negative relationship). Dijkstra et al. (2011), using a larger variety of diets (3 experiments, 10 dietary treatments) in which fat supplementation was a major source of dietary variation, evaluated relationships between CH₄ yield per unit of feed and milk FA profile in dairy cattle. Their prediction equation included milk FA concentration of C17:0-*anteiso* and *cis*-13 C18:1 (positive relationship) and *trans*-10+11 C18:1 and *cis*-11 C18:1 (negative relationship). The various models to predict CH₄ emission in these 3 studies have only a few milk FA in common. This might be a result of the small num-

ber of experiments and the limited variation in dietary treatments as well as the analytical methods used to elucidate milk FA profile. Data from a greater number of experiments containing a wider variety of diets are required to firmly assess the potential of milk FA profile as an indicator of CH₄ yield. A greater number of experiments also allows quantification of between-study variability or heterogeneity of the correlation between milk FA concentrations and CH₄ yield.

The aims of this study were to perform a meta-analysis to quantify relationships between CH₄ yield and individual milk FA concentrations in lactating dairy cattle while quantifying the heterogeneity of these relationships, and to develop equations to predict CH₄ yield (per unit of feed and per unit of milk) based on milk FA profile of cows fed a wide variety of diets. Such equations may ultimately be used to estimate CH₄ yield from dairy cattle under field conditions to fulfill the need for simple, inexpensive measurement techniques.

MATERIALS AND METHODS

Data Collection

For inclusion in the present meta-analysis, studies were required to have CH₄ production measured using respiration chambers and milk FA profile elucidated using gas chromatography. Four studies designed as 4 × 4 Latin squares from the University of Reading (Reading, UK) and 4 studies designed as randomized block experiments from Wageningen University (Wageningen, the Netherlands) met these requirements and were included (Table 1). The procedures to determine CH₄ production and milk FA profile are described by Kliem et al. (2008) and Reynolds et al. (2014) for the Reading studies and by van Knegsel et al. (2007) for the Wageningen studies. The 8 studies represented 30 different dietary treatments and 146 individual observations encompassing a variety of diets. Studies 1, 3, 4, 7, and 8 contained lipid treatments, whereas studies 2, 5, and 6 did not contain any lipid treatment. Studies 4 and 5 comprised diet treatments with different forage types and contents, and studies 2, 6, 7, and 8 contained various nonlipid additives. Animals were described by treatment diet composition, DMI, milk yield, milk composition, milk FA profile, and CH₄ production (Tables 2 and 3). Methane yield was expressed per unit of feed (g/kg of DMI) and per unit of fat- and protein-corrected milk (g/kg of FPCM), where FPCM (kg/d) = [0.337 + 0.116 × milk fat (%) + 0.06 × milk protein (%)] × milk production (kg/d) (CVB, 2008).

Some of the milk FA profile analyses did not allow identification of certain individual milk FA but did identify certain FA together as one fraction. When

Table 1. Data sources and characteristics of included studies

Study	Reference	n ¹	No. of treatments	Diet composition/treatment ²
1	University of Reading, UK Crompton et al. (2010)	16	4	Basal diet comprising 37.5% corn silage, 12.5% grass silage; treatments comprising 3.5% DM: control diet with calcium salts of palm oil fed once daily vs. control diet with calcium salts of palm oil fed twice daily vs. milled rapeseed diet fed twice daily vs. coconut oil diet fed twice daily.
2	Reynolds et al. (2010)	16	4	Basal diet comprising 37.5% grass silage, 12.5% corn silage; 50% concentrates vs. 50% concentrates + allicin vs. 40% concentrate + 10% glycerol vs. 20% concentrates + 30% naked oats.
3	Crompton et al. (2011) ³	16	4	Basal diet: 37.5% corn silage, 12.5% grass silage, 50% blend; 50:50 forage:concentrate ratio, no added lipid, diet fed twice daily vs. 50:50 forage:concentrate ratio, 3.5% lipid from milled rapeseed, diet fed twice daily vs. forage:concentrate ratio 50:50, 3.5% lipid from milled rapeseed, diet fed once daily vs. forage:concentrate ratio daily alternating 45:55 and 55:45, 3.5% lipid from milled rapeseed, diet fed once daily.
4	Reynolds et al. (2012)	16	4	Corn silage, grass silage, concentrates, extruded linseed (37.5:12.5:50.0:0.0 vs. 37.5:12.5:45.0:5.0 vs. 12.5:37.5:50.0:0.0 vs. 12.5:37.5:45.0:5.0).
5	Wageningen University, the Netherlands van Gastelen et al. (2014)	32	4	80% (100:0 vs. 67:33 vs. 33:67 vs. 0:100 grass silage:corn silage), 20% concentrates.
6	van Zijderveld et al. (2011a)	20	4	40% grass silage, 26% corn silage, 34% concentrates; control vs. diallyldisulfide supplement vs. yucca plant powder supplement vs. calcium fumarate supplement.
7	van Zijderveld et al. (2011b)	10	2	29% grass silage, 22% corn silage, 2% wheat straw, 47% concentrates; supplemented with palm oil vs. supplemented with calcium fumarate plus mix of lauric acid, myristic acid and linseed oil.
8	van Zijderveld et al. (2011a)	20	4	41% grass silage, 35% corn silage and 24% concentrates; control vs. extruded linseed supplement vs. diallyldisulfide supplement vs. mixture of caprylic acid and capric acid supplement.

¹Total number of observations.²Percentages on DM basis.³Experimental setup and description of diets in Relling et al. (2014).

Table 2. Descriptive statistics of dietary and animal characteristics

Variable	Mean	SD	Minimum	Maximum
DMI (kg/d)	18.5	2.41	13.1	26.1
NDF (% of DM)	37.0	3.76	29.0	42.2
ADF ¹ (% of DM)	21.7	2.05	17.9	27.6
Starch ² (% of DM)	16.5	6.39	0.42	25.7
Crude fat ³ (% of DM)	4.2	1.65	1.9	6.4
CP (% of DM)	16.0	1.12	13.3	19.1
Ash (% of DM)	7.3	0.92	5.0	9.4
Milk yield (kg/d)	28.9	6.40	16.8	44.4
FPCM ⁴ (kg/d)	29.1	5.14	18.3	42.4
Milk fat (g/100 g of milk)	4.20	0.679	2.28	6.24
Milk protein (g/100 g of milk)	3.29	0.314	2.38	4.18
Milk lactose (g/100 g of milk) ²	4.53	0.203	3.81	5.06
CH ₄ (g/d)	395	51.2	250	508
CH ₄ (g/kg of DMI)	21.5	2.46	15.9	27.9
CH ₄ (g/kg of FPCM)	13.9	2.30	8.8	20.3

¹No data available for experiments 6, 7, and 8.

²No data available for experiments 6 and 8.

³No data available for experiment 4.

⁴Fat- and protein-corrected milk.

these FA were individually identified in other studies, they were grouped together. Milk FA fractions were expressed in g/100 g of total milk FA. Fatty acid fractions with an average study concentration <0.1 g/100 g of milk FA were excluded from the data set.

Statistical Analysis

Random-Effects Model Analysis. Relationships between CH₄ yield per unit of feed and per unit of

milk, and individual milk FA concentrations for dairy cattle were meta-analyzed using the metafor package (version 1.6-0; Viechtbauer, 2010) in R (version 2.15.2; R Foundation for Statistical Computing, Vienna, Austria). The effect size of these relationships for each of the 8 studies was estimated by correlation coefficients (values in Supplemental Tables S1 and S2; <http://dx.doi.org/10.3168/jds.2014-8268>). The correlation coefficients were obtained by linear regression using individual animal data. In contrast to treatment mean

Table 3. Descriptive statistics of milk FA composition (g/100 g of total FA)

Milk FA	Mean	SD	Minimum	Maximum
C4:0	3.18	0.46	1.44	4.32
C6:0	2.10	0.35	0.73	2.73
C8:0	1.19	0.22	0.51	1.61
C10:0	2.67	0.57	1.12	3.77
C12:0	3.29	1.13	1.50	10.70
C14:0- <i>iso</i> ¹	0.10	0.04	0.03	0.22
C14:0	11.18	1.79	6.82	18.24
<i>cis</i> -9 C14:1	1.05	0.35	0.57	3.23
C15:0- <i>anteiso</i>	0.43	0.06	0.30	0.62
C15:0	0.99	0.25	0.64	2.25
C16:0- <i>iso</i>	0.22	0.07	0.01	0.37
C16:0	31.28	4.91	19.91	42.29
C17:0	0.55	0.12	0.27	0.82
C18:0	9.75	2.41	5.03	17.09
<i>trans</i> -6+7+8+9 C18:1 ²	0.57	0.32	0.25	1.63
<i>trans</i> -10+11 C18:1	1.46	1.01	0.51	9.00
<i>cis</i> -9 C18:1 ³	19.31	3.67	12.32	29.80
<i>cis</i> -11 C18:1 ²	0.60	0.21	0.30	1.37
<i>cis</i> -12 C18:1	0.28	0.13	0.07	0.81
<i>cis</i> -13 C18:1	0.17	0.11	0.04	0.65
<i>trans</i> -16+ <i>cis</i> -14 C18:1 ²	0.33	0.19	0.10	0.90
<i>cis</i> -9,12 C18:2	1.54	0.37	0.57	2.94
<i>cis</i> -9,12,15 C18:3	0.45	0.17	0.14	1.02
C20:0	0.13	0.04	0.06	0.24

¹No data available from experiment 7.

²No data available from experiment 5.

³Co-eluted with *trans*-13+14-C18:1 in experiments 1 and 3; co-eluted with *trans*-12-C18:1 in experiment 5.

data, use of individual animal data prevents one from ignoring variation of CH₄ production and milk FA concentrations at the animal level due to, for example, parity and DMI level. The correlation coefficients were transformed via Fisher's r-to-Z transformation, $Z = \tanh^{-1}(r)$, where \tanh^{-1} = the inverse hyperbolic tangent, which is defined as follows: $\tanh^{-1}(r) = 0.5 \times \ln[(1 + r)/(1 - r)]$ (Fisher, 1921). This transformation ensures more stable variance and normality. To obtain the average true effect, the meta-analytic model applied is given by

$$y_i = \mu + u_i + e_i, \quad [1]$$

where y_i is the observed effect in the i th study, μ is the average true effect, u_i is the variability among the true effect induced by study, and e_i is the sampling error with $e_i \sim N(0, v_i)$. The sampling variance, v_i , is known based on the number of observations per study. The study effect, u_i , was taken into account as a random factor. This model is referred to as the random-effects model.

In the random-effects model, the variability among studies (or heterogeneity), which is regarded to be induced by experimental circumstances, is assumed to be normally distributed with variance τ^2 such that $u_i \sim N(0, \tau^2)$. The model was fit with REML. Heterogeneity (τ^2) was expressed as percentage of the total variability in the effect size (τ^2 plus sampling error), yielding the I^2 statistic (Higgins et al., 2003). In case of negative values of I^2 , a value of 0% was adopted; I^2 values greater than 50% indicate substantial heterogeneity. The transformed correlations were tested for homogeneity with the Q-statistic (Hedges and Olkin, 1985), which follows a χ^2 distribution. Average true correlation coefficients and their boundaries of the 95% CI were back-transformed to raw correlation values for convenience of interpretation. Estimates of average true correlations were declared significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. The strength of the estimated average correlations is interpreted as small if $0.10 \leq |r| < 0.30$, medium if $0.30 \leq |r| < 0.50$, and large if $|r| \geq 0.50$ (Cohen, 1988).

Mixed Model Regression Analysis. To predict the actual CH₄ yield per unit of feed and per unit of milk, with milk FA concentrations as input, mixed model regression techniques (St-Pierre, 2001) were applied using PROC MIXED (SAS Institute Inc., Cary, NC). This enabled analysis of fixed effects of independent variables as well as the effect of study, which was taken into account as a random factor. The general model for single and multiple regression is represented as

$$Y_{ij} = B_0 + B_1 X_{ij1} + \dots + B_k X_{ijk} + s_i + e_{ij}, \quad [2]$$

where Y_{ij} is the dependent variable (i th study 1, ..., 8, j th observation 1, ..., 146) and X_{ijk} is the value of the k th explanatory variable ($k = 1, 2, \dots, p$). The overall intercept B_0 and the overall regression coefficients of Y on X across all studies comprise the fixed-effects part of the model for k different parameters. The random effect of the i th study on the overall intercept B_0 , s_i , together with the unexplained residual error, e_{ij} , comprise the random-effects part of the model, with both assumed to be normal. Random effects were modeled with (co) variance matrices that were fitted with an unstructured approach, providing that matrices converged. In cases of nonconvergence, (co)variance matrices were fitted with a compound symmetry. When matrices still did not converge, they were fitted with variance components. No random effect on slope was included in the multiple regression analysis to prevent overparameterization. A selection procedure for multiple regression was performed using a stepwise procedure (PROC GLMSELECT in SAS), retaining the experiment effect in every step, with CH₄ yield being the dependent variable and stepwise selection of FA based on the Schwarz Bayesian information criterion, where lower values indicate better model adequacy. All available single FA or FA combinations were included in the selection. For predicting CH₄ yield per unit of feed, milk fat and milk protein contents (g/100 g of milk) were included in the selection as well. Parameter estimates for fixed effects were declared significant at $P \leq 0.05$. Adjusted dependent variable values were calculated based on regression parameters of the final model to determine r or R^2 values corrected for experiment effect (St-Pierre, 2001). The residuals (predicted minus observed) were visually inspected for any patterns, as well as for any potentially confounding factors.

RESULTS AND DISCUSSION

The studies used in this meta-analysis (see Table 1) comprised a notably larger variety of diets compared with Chilliard et al. (2009), Mohammed et al. (2011), and Dijkstra et al. (2011). In the present data set, the forage proportion varied between 50 and 80% of total diet, with forage consisting of grass silage and corn silage in ratios ranging from 0:100 to 100:0 (all on a DM basis). It remains questionable, though, to what extent the present data set represents the variety of diets supplied on commercial dairy farms. In particular, the large variation in forage proportion (fraction of total diet) and composition (type of forage and quality of forage) in practice is not completely represented in the 8 studies included. As in the previous analyses cited above, the data used in the present study include measurements for diets that include supplemental lip-

ids and other ingredients with potential to reduce CH₄ yield.

Study effect can be taken into account as either a fixed or random factor in meta-analysis and leads to a fixed-effects model or a random-effects model, respectively. In contrast to fixed-effects models that make a conditional inference only about the number of studies included in the analysis, random-effects models estimate the unconditional inference about a larger set of studies of which the studies included in the analysis are assumed to be a normally distributed random sample (Viechtbauer, 2010). Therefore, including the study effect as a random factor, not as fixed factor, is in better agreement with the aim of this meta-analysis to search for a generally applicable prediction equation for CH₄ yield with milk FA concentration as input.

Random-Effects Model Analysis Correlation per Unit of Feed

The concentration of C16:0 in milk fat was moderately positively related to CH₄ yield (g/kg of DMI), and concentrations of C6:0, C8:0, and C10:0 in milk fat tended to be weakly positively related to CH₄ yield (Table 4), which is largely in agreement with previous findings (Chilliard et al., 2009). These FA are synthesized de novo in the mammary gland from acetate and β -hydroxybutyrate produced in the rumen (Bernard et al., 2008). Ruminant acetate production is positively associated with fiber intake (Bannink et al., 2008), which subsequently yields H₂ and ultimately CH₄ by methanogenic archaea. Milk C4:0 concentration was not significantly related to CH₄ yield. With the exception of C4:0, de novo FA synthesis in the mammary gland of C16 and shorter FA is inhibited in the presence of unsaturated long-chain FA (Bernard et al., 2008; Shingfield et al., 2010). The fact that dietary unsaturated FA generally reduce CH₄ yield may explain why concentrations of C4:0 were not related to CH₄ yield, in contrast to other even-chain de novo synthesized FA. Moreover, C4:0 in milk fat does not require acetate for its production as it can be produced directly from β -hydroxybutyrate derived from the blood and thus only partly originates from de novo FA synthesis using acetate.

As for milk FA concentration of C4:0, no significant relationship was found between CH₄ yield (g/kg of DMI) and concentrations of C12:0 or C14:0. Based on genetic and herd clustering of milk FA, Heck et al. (2012) found that C4:0 and C12:0 differed from the general pattern of the other FA in the group of de novo synthesized even-chain FA. Milk FA C12:0 and C14:0 are not only synthesized de novo in the mammary gland, but can also originate from dietary C12:0 and C14:0 (e.g., van Zijderveld et al., 2011b). Ingredients such as palm

kernel expeller and extracted coconut, with relatively large proportions of C12:0 and C14:0 in fat, are commonly included in dairy cattle diets and were present in various diets in the current data set. Dietary C12:0 and C14:0 inhibit CH₄ production (Patra, 2013), which might explain why no overall correlation of concentrations C12:0 and C14:0 in milk fat and CH₄ yield was found in this study. However, it should be noted that the level of either C12:0 or C14:0 fed was not available for most of the studies included in this meta-analysis. A part of C14:0 is desaturated to *cis*-9 C14:1 by Δ^9 -desaturation in the mammary gland. This desaturation activity is regulated by genetics (Soyeurt et al., 2008) and may be stimulated by acetate from the rumen and inhibited by unsaturated FA from feed (Chilliard et al., 2007; Jacobs et al., 2011). These contrasting mechanisms together may result in milk *cis*-9 C14:1 not being related to CH₄ yield in the present study. In contrast with our study, Chilliard et al. (2009) found milk FA concentrations of all even-chain de novo synthesized FA between C4:0 and C16:0 to be positively related to CH₄ production. However, in the study of Chilliard et al. (2009), dietary treatments differed only in physical form of linseed.

Concentrations of OBCFA in milk FA are related to molar proportions of VFA in the rumen and have potential to be used as rumen microbial markers or indicators of the type of VFA formed and as predictors of CH₄ yield (Vlaeminck et al., 2006a,b). Of the various OBCFA in milk fat in the present study, only C16:0-*iso* tended to be weakly positively related ($r = 0.22$) with CH₄ yield (g/kg of DMI). This weak positive relationship is in line with Mohammed et al. (2011) and Castro Montoya et al. (2011), who attributed this to the fact that *iso*-FA are more abundant in cellulolytic bacteria (Vlaeminck et al., 2006a), which are associated with higher CH₄ yield. Milk C14:0-*iso* concentration was positively related to CH₄ yield in the study of Chilliard et al. (2009). An increased level of fiber in the diet generally results in increased CH₄ yield and is associated with increased concentrations of C14:0-*iso* in milk fat (Boivin et al., 2013). This positive relationship was not confirmed in the current meta-analysis ($P = 0.131$). Vlaeminck et al. (2006a) reported negative and positive correlations of milk C15:0-*anteiso* concentration with NDF and starch, respectively. These 2 feed components have counteracting effects on CH₄ yield (Ellis et al., 2008), which might explain why the concentration C15:0-*anteiso* in milk fat was not significantly related to CH₄ yield in the present study. This result is in line with Fievez et al. (2012), who suggested that the concentration of C15:0-*anteiso* was relevant only in prediction of butyrate proportion in the rumen that is associated with dietary sugars (Oba, 2011). Elevated

Table 4. Estimated true correlation coefficients with standard errors and *P*-values, *P*-values for heterogeneity test statistic (Q-value *P*), and heterogeneity as a fraction of total variability (\hat{I}^2) for the correlation between CH₄ yield per unit of feed and per unit of milk (fat- and protein-corrected milk, FPCM), and milk FA concentration

Milk FA	CH ₄ yield (g/kg of DMI) vs. milk FA concentration					CH ₄ yield (g/kg of FPCM) vs. milk FA concentration				
	r	SE	<i>P</i> -value	Q-value <i>P</i>	\hat{I}^2 (%)	r	SE	<i>P</i> -value	Q-value <i>P</i>	\hat{I}^2 (%)
C4:0	0.07	0.147	0.647	0.016	60.7	-0.28	0.090	0.001	0.358	0.0
C6:0	0.19	0.103	0.056	0.265	21.0	0.02	0.090	0.830	0.837	0.0
C8:0	0.23	0.127	0.066	0.072	47.5	0.15	0.095	0.119	0.404	8.5
C10:0	0.18	0.096	0.057	0.381	10.7	0.24	0.105	0.021	0.258	23.7
C12:0	0.03	0.182	0.878	0.001	74.9	0.36	0.113	0.001	0.177	34.2
C14:0- <i>iso</i>	0.26	0.173	0.131	<0.001	70.5	0.34	0.205	0.093	<0.001	79.1
C14:0	-0.02	0.194	0.902	0.001	77.8	0.26	0.115	0.021	0.152	36.3
<i>cis</i> -9 C14:1	-0.17	0.152	0.257	0.012	63.5	0.27	0.097	0.004	0.365	11.2
C15:0- <i>anteiso</i>	-0.19	0.185	0.316	0.001	75.6	0.10	0.151	0.526	0.010	62.9
C15:0	-0.16	0.190	0.414	0.000	76.9	0.28	0.139	0.043	0.028	55.9
C16:0- <i>iso</i>	0.22	0.129	0.079	0.056	49.2	0.12	0.120	0.337	0.104	41.5
C16:0	0.34	0.112	0.001	0.080	33.1	0.29	0.090	0.001	0.904	0.0
C17:0	0.11	0.161	0.490	0.005	67.5	-0.09	0.114	0.454	0.139	35.5
C18:0	0.07	0.108	0.527	0.201	28.2	-0.24	0.090	0.006	0.530	0.0
<i>trans</i> -6+7+8+9 C18:1	-0.27	0.103	0.008	0.557	0.0	-0.16	0.109	0.154	0.410	10.1
<i>trans</i> -10+11 C18:1	-0.56	0.157	<0.001	0.005	65.7	-0.16	0.090	0.068	0.869	0.0
<i>cis</i> -9 C18:1	-0.13	0.157	0.412	0.007	65.7	-0.41	0.090	<0.001	0.896	0.0
<i>cis</i> -11 C18:1	-0.52	0.151	<0.001	0.045	52.3	-0.45	0.155	0.002	0.037	55.0
<i>cis</i> -12 C18:1	-0.40	0.192	0.030	<0.001	77.5	-0.13	0.090	0.152	0.970	0.0
<i>cis</i> -13 C18:1	-0.26	0.141	0.057	0.024	57.3	-0.13	0.123	0.307	0.086	44.4
<i>trans</i> -16+ <i>cis</i> -14 C18:1	-0.35	0.214	0.088	0.001	76.7	-0.15	0.135	0.253	0.127	40.8
<i>cis</i> -9,12 C18:2	-0.25	0.143	0.082	0.021	58.9	-0.28	0.090	0.001	0.432	0.0
<i>cis</i> -9,12,15 C18:3	0.00	0.090	0.978	0.642	0.0	-0.12	0.113	0.300	0.145	34.3
C20:0	-0.02	0.148	0.871	0.020	57.4	-0.14	0.123	0.268	0.121	38.9

sugar contents may not increase CH₄ yield (Staerfl et al., 2012) and may indicate that CH₄ yield and milk C15:0-*anteiso* concentration are not related.

No significant relationships with CH₄ yield (g/kg of DMI) were found for concentrations of C15:0 and C17:0 in milk fat (Table 4). Two different hypotheses on the relationship between diet composition and these odd-chain milk FA concentrations have been investigated. Patel et al. (2013) reported increased milk C15:0 concentration when feeding increased proportions of grass silage and dietary NDF levels, which was attributed to more abundant membrane lipids from rumen microbes. A high NDF content in the feed is associated with increased CH₄ yield (Ellis et al., 2008). This indicates a positive relationship between odd-chain milk FA concentrations and CH₄ yield. In contrast, Castro Montoya et al. (2011) reported milk concentrations of C15:0 and the sum of C17:0 and *cis*-9 C17:1 to be positively related to propionate concentration in the rumen as these are synthesized from propionate de novo (French et al., 2012). Propionate production is negatively related to CH₄ production, suggesting a negative relationship between milk odd-chain FA concentration and CH₄ yield. In the present meta-analysis, odd-chain FA concentrations in milk fat were not significantly related to CH₄ yield. It should be noted that the concentration of *cis*-9 C17:1, a desaturation product of C17:0 in the

mammary gland, was not available in all experiments included in the present study. Grouping these 2 fractions together based on a broad database might shed new light on the relationship between CH₄ yield and odd-chain FA concentration in milk, as also reported by Dijkstra et al. (2011), where the sum of milk FA concentrations C17:0 and *cis*-9 C17:1 was negatively related to CH₄ yield. Overall, in line with Chilliard et al. (2009) and Mohammed et al. (2011), relationships between concentrations of OBCFA in milk and CH₄ production were generally rather minor or absent, and also less than expected based on theoretical relationships between rumen fermentation products and CH₄ yield (e.g., Vlaeminck et al., 2006a,b; Castro Montoya et al., 2011).

Several long-chain unsaturated FA in milk originate from dietary oils and their biohydrogenation products formed in the rumen. Higher concentrations of these FA in cattle diets, which are known to reduce DM and NDF digestibility, are negatively associated with CH₄ yield (e.g., Patra, 2013). Milk *cis*-9,12 C18:2 and *cis*-9,12,15 C18:3 directly originate from the corresponding FA in feed (Chilliard et al., 2007). Concentration of *cis*-9,12 C18:2 in milk fat tended to be negatively related to CH₄ yield (g/kg of DMI), $r = -0.25$, in line with expectations. In general, replacing grass silage with corn silage reduces CH₄ yield, and corn silage is rich

in *cis*-9,12 C18:2 and increases the proportion of this FA in milk fat (Kliem et al., 2008). However, no relationship between milk *cis*-9,12,15 C18:3 concentration and CH₄ yield was found in the present meta-analysis. Chilliard et al. (2007) stated that the potential to increase milk FA concentration *cis*-9,12,15 C18:3 was limited, with some positive effects upon feeding protected oilseed supplements in particular. Oilseeds in protected form may not decrease methanogenesis (Dohme et al., 2000) and may not result in a significant negative correlation between CH₄ yield and milk *cis*-9,12,15 C18:3 concentration. The absence of a significant relationship between milk *cis*-9,12,15 C18:3 concentration and CH₄ yield is in line with Chilliard et al. (2009) for diets that differ in type of linseed supplemented. Nonetheless, the study of Mohammed et al. (2011) included a linseed treatment, which is high in *cis*-9,12,15 C18:3, and indicated a moderate negative correlation between concentration of *cis*-9,12,15 C18:3 in milk fat and CH₄ production (g/d). Additional evaluation of the rumen microbial metabolism of *cis*-9,12,15 C18:3 thus seems to be necessary to better understand these contrasting findings.

Various *trans*- and *cis*-C18:1 milk FA concentrations were negatively related to CH₄ yield (g/kg of DMI), as shown in Table 4. In general, *trans*-11 C18:1 is the major monounsaturated biohydrogenation intermediate of both *cis*-9,12,15 C18:3 and *cis*-9,12 C18:2 (e.g., Shingfield et al., 2010). These PUFA inhibit CH₄ production, and milk *trans*-11 C18:1 concentration is therefore expected to be negatively related to CH₄ yield. With reduced rumen pH values, the predominant biohydrogenation pathway of *cis*-9,12 C18:2 may shift to *trans*-10 C18:1 (Colman et al., 2012) and low rumen pH is negatively associated with CH₄ production (Ellis et al., 2008). Besides, diets rich in unsaturated FA often cause a shift to *trans*-10 C18:1 formation (Mohammed et al., 2011) when 70% of the diet is roughage (Boeckaert et al., 2008). These observations explain the strong negative correlation ($r = -0.56$) obtained between milk *trans*-10+11 C18:1 concentration and CH₄ yield. Like *trans*-10+11 C18:1, *cis*-11 C18:1 and *cis*-12 C18:1 and the fraction *trans*-6+7+8+9 C18:1 result from biohydrogenation of both *cis*-9,12 C18:2 and *cis*-9,12,15 C18:3 (e.g., Jouany et al., 2007; Shingfield et al., 2010), and this explains their significantly negative strong ($r = -0.53$; *cis*-11 C18:1), moderate ($r = -0.40$; *cis*-12 C18:1), and weak ($r = -0.27$; *trans*-6+7+8+9 C18:1) relationship of concentrations of these FA with CH₄ yield.

Depending on their FA composition, different dietary lipids result in variable biohydrogenation products. Milk *trans*-16+*cis*-14 C18:1 concentration tended to be moderately negative related to CH₄ yield ($r = -0.35$). Elevated levels of *trans*-16 C18:1 have been found in

duodenal digesta (Glasser et al., 2008) and in milk (Kliem et al., 2009) upon supplementing diets with feed ingredients rich in *cis*-9,12,15 C18:3. *trans*-16+*cis*-14 C18:1 also appeared as an in vitro rumen biohydrogenation product of *cis*-9,12,15 C18:3 (Jouany et al., 2007), which might suggest that milk *cis*-14 C18:1 is derived from *cis*-9,12,15 C18:3. *cis*-13 C18:1 was increased in milk when increased contents of corn silage were fed (Kliem et al., 2008) and was increased in vitro when *cis*-9,12 C18:2 or *cis*-9,12,15 C18:3 were used as a substrate (Jouany et al., 2007). In this meta-analysis, the relationship tended to be weakly negative ($r = -0.26$) and may suggest that *cis*-13 C18:1 is derived from both *cis*-9,12 C18:2 and *cis*-9,12,15 C18:3. The positive response of milk *cis*-13 C18:1 concentration to dietary linseed oil supplementation (Loor et al., 2004) further supports this.

Milk *cis*-9 C18:1 concentration was not significantly related to CH₄ yield in the present meta-analysis. The absence of a significant relationship is in line with the analysis of Mohammed et al. (2011), whereas Chilliard et al. (2009) did find a significantly negative relationship. *cis*-9 C18:1, which inhibits CH₄ production (e.g., Patra, 2013), is present in many feedstuffs and might be less sensitive to biohydrogenation than other unsaturated FA in high concentrate diets (Loor et al., 2004). Nonetheless, *cis*-9 C18:1 is converted into *trans*-C18:1 isomers in the rumen, possibly contributing to the negative correlations between concentrations of several milk *trans*-C18:1 isomers and CH₄ yield as obtained in this study and discussed earlier (e.g., Shingfield et al. 2010). Δ^9 -Desaturation of C18:0 in the mammary gland is another mechanism by which *cis*-9 C18:1 appears in milk. Furthermore, dietary C18:0, being a possible substrate for Δ^9 -desaturase, was not observed to inhibit CH₄ production (Patra, 2013), so does not contribute to a relation of milk *cis*-9 C18:1 and C18:0 concentrations with CH₄ yield. In line with this and Mohammed et al. (2011), no relationship between milk C18:0 concentration and CH₄ yield was found in this meta-analysis. This is in contrast with Chilliard et al. (2009), who found a negative relationship, possibly coming from dietary linoleic and linolenic acid, which were biohydrogenated to C18:0 that was absorbed. Milk C20:0 is an elongation product by action of elongase enzymes on C18:0 from the diet or from body fat. The fact that milk C20:0 concentration and CH₄ yield were not related in the present meta-analysis is in line with C18:0 concentration not being related overall to CH₄ yield.

Evaluation of Heterogeneity

Milk C6:0, C8:0, C10:0, and C16:0 concentrations were not substantially heterogeneously correlated to

CH₄ yield per unit of feed (Table 4). These relatively low heterogeneities may indicate good precision of the estimated relationships and the simplicity of the mechanism determining the relationship. Next to de novo FA synthesis and feed, body fat is another source of milk C16:0 (Gross et al., 2011). Nonetheless, the de novo FA synthesis that regulates milk C16:0 concentration is positively associated with CH₄ yield. Milk C16:0 from body fat and feed may not induce heterogeneity among the relationship. Unlike that of other saturated even-chain FA, milk C12:0 and C14:0 concentrations showed substantial heterogeneity among their true correlation with CH₄ yield. Variation in diet composition, in particular when ingredients relatively rich in C12:0 and C14:0 are supplied to cattle (discussed in a previous section), may explain such heterogeneity. The sum of all de novo synthesized even-chain FA concentrations (C4:0 to C16:0, including C12:0 and C14:0) did not show any heterogeneity among its true correlation (Table 5).

The correlation of milk C14:0-*iso*, C15:0-*anteiso*, C15:0, C17:0 (Table 4), and of combined OBCFA concentrations; namely, C14:0-*iso*+C16:0-*iso* and C15:0+C17:0 (Table 5), were all substantially heterogeneous ($I^2 \geq 61.1\%$). The heterogeneity observed for these milk OBCFA concentrations might reflect the variation in microbial species and activities in the rumen. All concentrations of individual C18:1 fractions appeared to be substantially heterogeneous ($I^2 > 50\%$), except for the *trans*-6+7+8+9 C18:1 fraction, which was totally homogeneous (Table 4). The combination of these 4 different FA may balance out all heterogeneity

but does not indicate homogeneity for the correlation of the 4 individual FA concentrations. The correlation of the concentration of a combined fraction of milk *cis*-11+12+13 C18:1 with CH₄ yield was also homogeneous, even though substantial heterogeneity was observed using the individual milk FA concentrations (Table 5). Substantial heterogeneity was apparent for correlations between CH₄ yield and concentrations of all combined fractions of C18:1 isomers that contained *trans*-10+11 C18:1 and a tendency for heterogeneity when *trans*-16+*cis*-14 C18:1 was added to the fraction *trans*-6+7+8+9 C18:1 + *cis*-11+12+13 C18:1 (Table 5). Various concentrations of fractions with milk *trans*-C18:1 isomers and *cis*-9,12 C18:2 ($I^2 = 59.0\%$) did not, therefore, appear to be precise indicators of CH₄ yield, whereas milk *cis*-11+12+13 C18:1 and *trans*-6+7+8+9 C18:1 concentration did. The C18:1 isomers in milk other than isomers containing a *cis*-9 double bond mainly originate from the rumen, but microorganisms and enzymes responsible for their production are not well characterized, and candidate bacterial species have yet to be cultivated (Wallace et al., 2007; Lourenço et al., 2010). Isolation of bacterial species may help to better interpret heterogeneity and homogeneity observed.

Random-Effects Model Analysis Correlation per Unit of Milk

Positive relationships were obtained between CH₄ yield (g/kg of FPCM) and the milk FA concentrations of C10:0, C12:0, C14:0, *cis*-9 C14:1, C15:0, and C16:0. The concentration of C14:0-*iso* tended to be

Table 5. Estimated true correlation coefficients with standard errors and *P*-values, *P*-values for heterogeneity test statistic (Q-value *P*), and heterogeneity as a fraction of total variability (I^2) for the correlation between CH₄ yield per unit of feed and milk FA concentration

Milk FA	r	SE	<i>P</i> -value	Q-value <i>P</i>	I^2 (%)
C6:0+C8:0+C10:0	0.22	0.11	0.050	0.173	34.0
C6:0+C8:0+C10:0+C16:0	0.34	0.10	<0.001	0.149	16.1
C12:0+C14:0	-0.02	0.20	0.919	<0.001	79.4
Even-chain C4:0 to C16:0	0.26	0.09	0.003	0.501	0.0
C16:0+C18:0	0.42	0.09	<0.001	0.128	5.6
C15:0+C17:0	-0.07	0.16	0.677	0.005	66.4
C14:0- <i>iso</i> +C16:0- <i>iso</i> ¹	0.24	0.15	0.108	0.012	61.1
<i>trans</i> -C18:1 ²	-0.52	0.18	<0.001	0.009	66.0
<i>cis</i> -C18:1 ³	-0.52	0.10	<0.001	0.570	0.0
C18:1 ⁴	-0.56	0.16	<0.001	0.003	68.5
C18:1 ⁵	-0.49	0.14	<0.001	0.079	42.1
C18:1 ⁶	-0.56	0.15	<0.001	0.045	52.5
C18:2+C18:3	-0.21	0.10	0.042	0.170	19.7

¹No data for experiment 7.

²*trans*-6+7+8+9+10+11-C18:1, no data for experiment 5.

³*cis*-11+12+13-C18:1, no data for experiment 5.

⁴*trans*-10+11-C18:1+*cis*-12+13-C18:1.

⁵*trans*-6+7+8+9+16-C18:1+*cis*-11+12+13+14-C18:1, no data for experiment 5.

⁶*trans*-6+7+8+9+10+11+16-C18:1+*cis*-11+12+13+14-C18:1, no data for experiment 5.

positively related to CH₄ yield. Negative relationships or a tendency for a negative relationship were obtained between CH₄ yield and the milk FA concentration of C18:0, *cis*-9 C18:1, *cis*-11 C18:1, *trans*-10+11 C18:1, and *cis*-9,12 C18:2. These relationships and the absence of significant relationships between CH₄ yield and milk FA concentrations of C17:0, *cis*-9,12,15 C18:3, and C20:0 are largely in agreement with the correlations obtained when CH₄ yield was expressed per unit of feed. In contrast to CH₄ yield per unit of feed, no positive relationships were obtained between CH₄ yield per unit of milk and milk FA concentration of C6:0 and C8:0, and a negative relationship was observed for the concentration of C4:0. Moreover, no significant relationship was obtained between the milk FA concentrations of *trans*-6+7+8+9 C18:1, *cis*-12 C18:1, *cis*-13 C18:1, and *trans*-16+*cis*-14 C18:1 and CH₄ yield per unit of milk. This might be due to the fact that various biohydrogenation intermediates associated with a reduction in CH₄ yield per unit feed (Table 4) are associated with milk fat depression (e.g., Piperova et al. 2000), which negatively affects the amount of FPCM, thus yielding more CH₄ yield per unit of milk. A reduced correlation strength for *trans*-10+11 C18:1 per unit of feed and per unit of milk ($r = -0.56$ vs. $r = -0.16$, respectively) is in line with this. The negative relationship between CH₄ yield per unit of milk and the concentration of C18:0 in milk fat may be explained by the concentration of C18:0 being decreased during lactation (Stoop et al., 2009), when milk yield decreases and next CH₄ yield per unit of milk increases. Milk FA concentration of the fraction odd-chain C5:0 to C15:0 and of *cis*-9 C18:1 are decreased and increased, respectively, during negative energy balance in early lactation (Stoop et al., 2009; Gross et al., 2011) when cows are producing at a high level. High milk production is associated with lower CH₄ yield per unit of milk, which may explain why milk FA concentrations of C15:0 and *cis*-9 C18:1 were positively and negatively related to CH₄ yield per unit of milk, as also shown by Chilliard et al. (2009). Concentrations of C15:0-*anteiso* and C16:0-*iso* in milk fat were not significantly related to CH₄ yield per unit of milk, possibly because the proportion of branched-chain FA does not vary during lactation (Stoop et al., 2009).

Most of the milk FA that were significantly correlated, or tended to be correlated, with CH₄ yield per unit of milk showed relatively low heterogeneity, with the exception of C14:0-*iso*, C15:0, and *cis*-11 C18:1 (Table 4). In general, milk FA concentrations may therefore be regarded as precise indicators of CH₄ yield per unit of milk.

Correlations between CH₄ yield (g/kg of FPCM) and combined milk FA concentrations were sig-

nificantly positive for C10:0+C12:0+C14:0, C10:0+C12:0+C14:0+C16:0, even-chain C4:0 to C16:0, and C16:0+C18:0, and were significantly negative or tended to be negative for the various combined fractions consisting of C18:1 isomers and for the sum of C18:2 and C18:3 (Table 6). The strongest positive and negative correlations were not stronger than those based on single FA fractions: 0.36 versus 0.36 for C10:0+C12:0+C14:0+C16:0 and C12:0, and -0.43 versus -0.45 for *trans*-10+11 C18:1+*cis*-9+11 C18:1 and *cis*-11 C18:1, respectively. The fractions of even-chain saturated FA and C18:1 isomers were not or not substantially heterogeneously correlated. Concentrations of combined fractions of milk OBCFA; namely, C15:0+C17:0 and C14:0-*iso*+C16:0-*iso*, were not related to CH₄ yield (g/kg of FPCM), which is in line with the absence of such relationships of CH₄ yield per unit feed. In general, most combined and single concentrations of milk FA showed less heterogeneity in the correlation with CH₄ yield per unit of milk than with CH₄ yield per unit of feed.

Mixed Model Regression Analysis for CH₄ Yield per Unit of Feed

Mixed model fits to evaluate the potential to predict CH₄ yield (g/kg of DMI) using selected milk FA concentrations (g/100 g of FA) resulted in R² values of 0.15 and 0.17 for the concentration of milk C16:0 and the fraction C6:0+C8:0+10:0+C16:0, respectively (Figure 1). Thus, the concentration of the fraction C6:0+C8:0+10:0+C16:0 did not show a substantially stronger positive relationship with CH₄ yield than the concentration of C16:0 alone. The R² values were 0.20, 0.41, 0.31, and 0.41 for the prediction with milk FA concentrations of *trans*-6+7+8+9 C18:1, *cis*-11 C18:1, *cis*-11+12+13 C18:1, and C18:1 (*trans*-6+7+8+9+10+11+16 C18:1+*cis*-11+12+13+14 C18:1), respectively. Concentrations of C18:1 fractions that were grouped together did not result in a substantially stronger negative relationship with CH₄ yield than concentrations of single C18:1 fractions.

The best multiple regression to predict CH₄ yield using concentrations of milk FA is:

$$\begin{aligned} \text{CH}_4 \text{ (g/kg of DMI)} = & 23.39 \pm 1.21 + 9.74 \pm 3.23 \\ & \times \text{C16:0-iso} - 1.06 \pm 0.17 \times \text{trans-10+11 C18:1} \\ & - 1.75 \pm 0.49 \times \text{cis-9,12 C18:2}, \end{aligned} \quad [3]$$

where milk FA concentrations are in g/100 g of total FA, R² = 0.54 after correction for experiment effect (St-Pierre, 2001), and $P \leq 0.003$ for all parameters. The

Table 6. Estimated true correlation coefficients with standard errors and *P*-values, *P*-values for heterogeneity test statistic (Q-value *P*), and heterogeneity as a fraction of total variability (*F*²) for the correlation between CH₄ yield per unit of milk and milk FA concentration

Milk FA	r	SE	<i>P</i> -value	Q-value <i>P</i>	<i>F</i> ² (%)
C6:0+C8:0	0.08	0.090	0.384	0.753	0.0
C10:0+C12:0+C14:0	0.30	0.112	0.006	0.182	33.1
C10:0+C12:0+C14:0+C16:0	0.36	0.090	<0.001	0.982	0.0
Even-chain C4:0 to C16:0	0.34	0.090	<0.001	0.979	0.0
C16:0+C18:0	0.23	0.090	0.010	0.794	0.0
C15:0+C17:0	0.23	0.154	0.128	0.009	64.3
C14:0- <i>iso</i> +C16:0- <i>iso</i> ¹	0.22	0.183	0.233	0.002	73.6
<i>trans</i> -C18:1 ²	-0.17	0.103	0.092	0.720	0.0
<i>cis</i> -C18:1 ³	-0.41	0.103	<0.001	0.809	0.0
C18:1 ⁴	-0.43	0.090	<0.001	0.864	0.0
C18:1 ⁵	-0.43	0.103	<0.001	0.735	0.0
C18:1 ⁶	-0.42	0.103	<0.001	0.734	0.0
C18:2+C18:3	-0.24	0.090	0.007	0.516	0.0

¹No data for experiment 7.²*trans*-6+7+8+9+10+11 C18:1, no data for experiment 5.³*cis*-9+11+12+13 C18:1, no data for experiment 5.⁴*trans*-10+11 C18:1+*cis*-9+12+13 C18:1.⁵*trans*-10+11 C18:1+*cis*-9+11 C18:1, no data for experiment 5.⁶*trans*-6+7+8+9+10+11+16 C18:1+*cis*-9+11+12+13+14 C18:1, no data for experiment 5.

model acceptably reproduced the CH₄ yield (Figure 2), as the slope and intercept of the regression between observed and predicted values did not differ from 1 and 0, respectively (results not shown). No clear patterns appear in the observed versus predicted values of CH₄ yield and residual versus predicted values of CH₄ yield (Figure 3), which does not indicate substantial bias or heteroscedasticity. The obtained R² was larger than for concentrations of single milk FA fractions but lower than the value (0.73) that Dijkstra et al. (2011) obtained for their equation based on 3 experiments, and also lower than 0.82 (single experiment; Mohammed et al., 2011; only FA concentrations included in prediction equation) and 0.95 (single experiment; Chilliard et al., 2009; milk FA concentration and forage intake in prediction equation). The data used by Dijkstra et al. (2011) are also included in the present analysis. The larger number of studies included in our meta-analysis, including a wider variety of diet compositions, may have resulted in the lower R² value of Equation [3]. A similar issue may hold when comparing the multiple regression analysis of Dijkstra et al. (2011) with the analyses of Mohammed et al. (2011), based on diets supplemented with sunflower seed, linseed, and canola seed, and Chilliard et al. (2009), based on linseed supplements only. On the other hand, Dijkstra et al. (2011) did not include forage intake in their equation and expressed CH₄ yield in grams per kilogram of DMI, whereas Chilliard et al. (2009) and Mohammed et al. (2011) expressed CH₄ production in grams per day. Furthermore, the substantial heterogeneity of the correlation of CH₄ yield and milk FA concentrations of several C18:1 isomers found

in the present analysis may limit a precise prediction of CH₄ yield (g/kg of DMI) using milk FA profile.

Equation [3] of the present meta-analysis has various milk FA concentrations in common with previously reported equations. Milk C16:0-*iso* concentration did appear in the best equation of Mohammed et al. (2011). Milk *trans*-10+11 C18:1 concentration was also included by Dijkstra et al. (2011), and Mohammed et al. (2011) included all *trans*-C18:1 FA concentrations in their second-best equation based on milk FA concentrations and DMI. Milk *cis*-9,12 C18:2 concentrations was also present in the best equation of Chilliard et al. (2009). However, milk *cis*-9,12 C18:2 concentration was positively related to CH₄ in that equation, whereas it was negatively related in Equation [3] of the current meta-analysis.

Different FA concentrations available for selection in different studies may hamper the development of a universally valid CH₄ prediction equation based on milk FA concentrations. A stepwise selection was performed for concentrations of all milk FA that were available in 7 experiments to extend the number of available FA with C14:0-*iso*, *trans*-6+7+8+9 C18:1, *cis*-11 C18:1, and *trans*-16+*cis*-14 C18:1. Methane yield was best predicted by the concentration of C16:0-*iso* (positively related) and the concentrations of *cis*-11 C18:1 and *trans*-16+*cis*-14 C18:1 (both negatively related), with *P* ≤ 0.02 for all regressors and R² = 0.55. The concentration of *cis*-11 C18:1 was also present in the equation of Dijkstra et al. (2011) and in the second-best equation of Mohammed et al. (2011), and *trans*-16+*cis*-14 C18:1 concentration also appeared in the best and second-

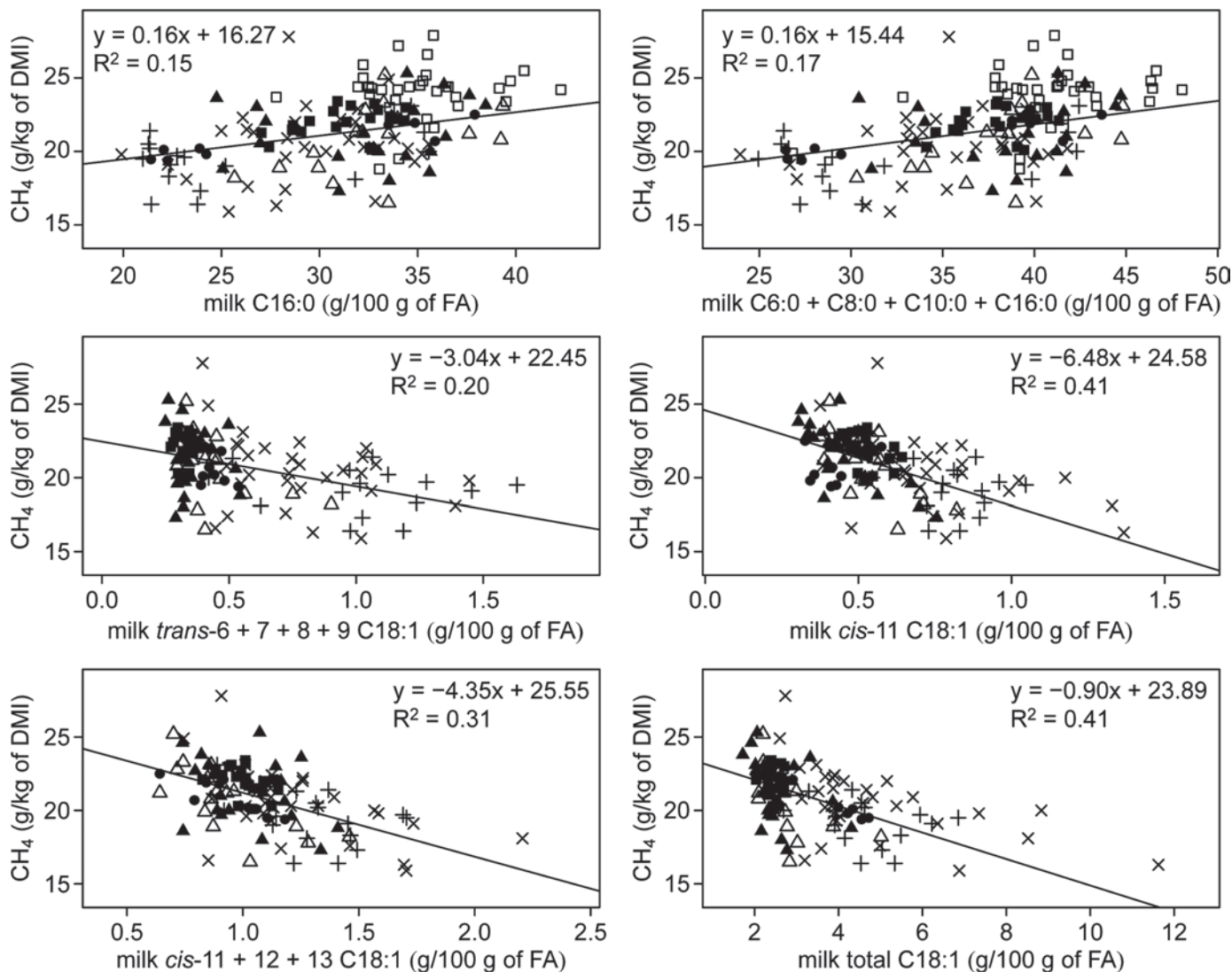


Figure 1. Relationships between CH₄ yield (g/kg of DMI) and selected milk FA concentrations. Milk total C18:1 comprises *trans*-6+7+8+9 C18:1, *trans*-10+11 C18:1, *cis*-11 C18:1, *cis*-12 C18:1, *cis*-13 C18:1, and *trans*-16+*cis*-14 C18:1 for experiments 1 to 4 and 6 to 8. The different symbols identify the 8 individual experiments described in Table 1 (see Figure 2 for explanation).

best equations of Chilliard et al. (2009). However, the equation based on 7 experiments with $R^2 = 0.55$ is not preferred over Equation [3] because it is based on one less experiment and the coefficient of determination is just marginally higher. Total milk fat and milk protein content were never selected and might illustrate the value of concentrations of single milk FA to predict CH₄ yield.

In addition to gas chromatography, Fourier transform infrared spectroscopy (FTIR) is often applied to quantify FA concentrations in milk. To achieve a reasonable accuracy of milk FA concentrations with FTIR, FA should have an average concentration of ≥ 2.45 g/100 g of FA (Rutten et al., 2009). Restricting the selection of milk FA with this threshold concentration would have

resulted in a best equation based on positive relationships of concentrations of C14:0, C16:0, and C18:0 and $R^2 = 0.29$. More recently, Soyeurt et al. (2011) found concentrations of milk C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, all *trans*-C18:1, *cis*-9 C18:1, all *cis*-C18:1, and some groups of FA in milk to be sufficiently accurately determined by FTIR to be used in milk payment systems. Including these FA concentrations mentioned by Soyeurt et al. (2011) in a selection procedure resulted in a best equation with a negative relationship of all *trans*-C18:1 concentration, where it is noted that the *trans*-C18:1 fraction consisted of all *trans*-C18:1 available in studies 1 to 4 and 6 to 8. The R^2 value was 0.43 for this equation, lower than that obtained for Equation [3]. Several milk FA with lower

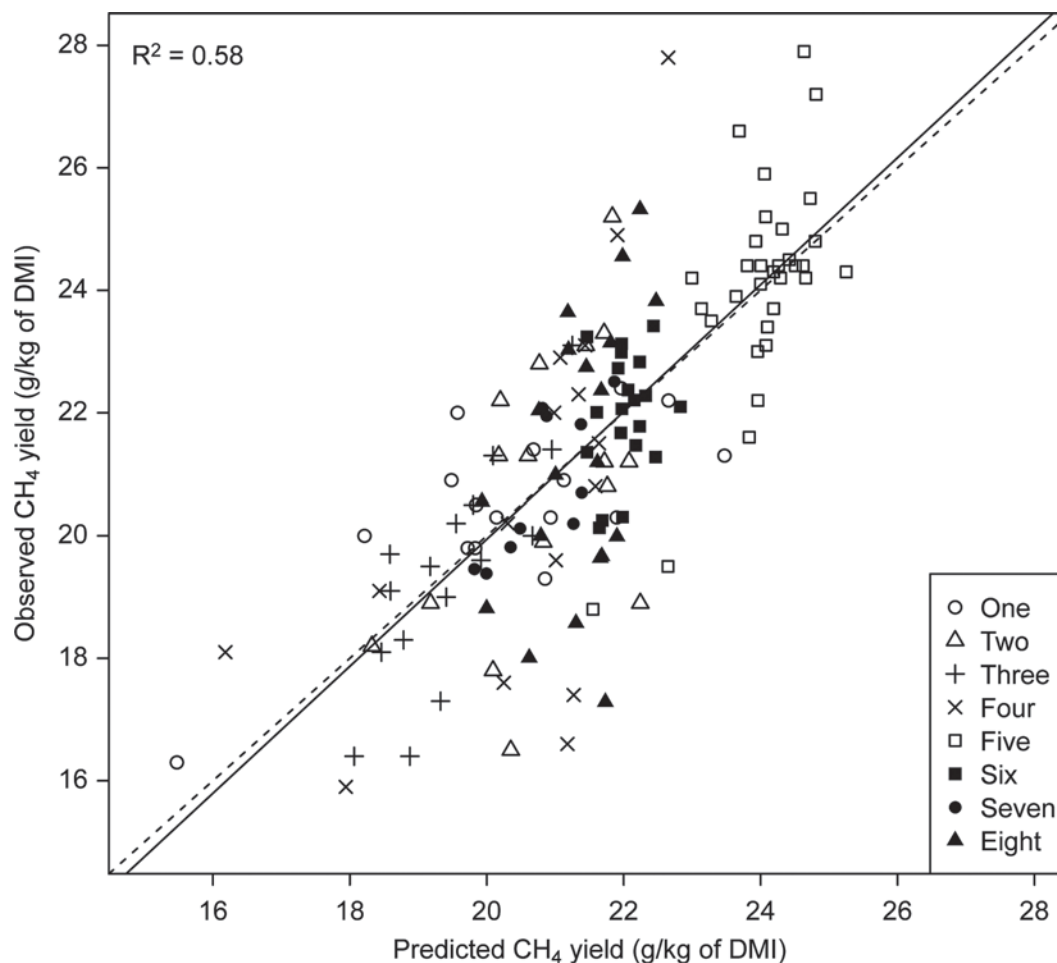


Figure 2. Observed versus predicted CH₄ yield per unit of feed from Equation [3], including experiment as a discrete class variable with experiment effect not shown. The different symbols identify the 8 individual experiments. The line of unit slope represents the line of equivalence.

concentrations that appear in various equations published previously are not available when milk FA profile is determined using FTIR. Furthermore, concentrations of combined FA fractions, several of which are accurately determined with FTIR, did not substantially increase the potential for predicting CH₄ yield (Figure 1). Compared with gas chromatography, the current performance of FTIR therefore limits the potential for predicting CH₄ yield based on milk FA profile.

Mixed Model Regression Analysis for CH₄ Yield per Unit of Milk

The best multiple regression to predict CH₄ yield using concentrations of milk FA was as follows:

$$\begin{aligned} \text{CH}_4 \text{ (g/kg of FPCM)} = & 21.13 \pm 1.72 - 1.38 \pm 0.38 \\ & \times \text{C4:0} + 8.53 \pm 3.05 \times \text{C16:0-iso} - 0.22 \pm 0.04 \\ & \times \text{cis-9 C18:1} - 0.59 \pm 0.18 \times \text{trans-10+11 C18:1}, \quad [4] \end{aligned}$$

where $R^2 = 0.47$ after correction for experiment effect (St-Pierre, 2001) and $P \leq 0.006$ for all parameters. The model acceptably reproduced the CH₄ yield (Figure 4), as the slope and intercept of the regression between observed and predicted values did not differ from 1 and 0, respectively (results not shown). No clear patterns appear in the observed versus predicted values of CH₄ yield and residual versus predicted values of CH₄ yield (Figure 5), which does not indicate substantial bias or heteroscedasticity. The obtained R^2 value was somewhat smaller than that for the prediction of CH₄ yield per unit of feed. Equation [4] also contains milk C16:0-*iso* and *trans*-10+11 C18:1 concentration (like Equation [3]) to predict CH₄ yield per unit of feed. Therefore, this may be regarded as further evidence for concentrations of milk C16:0-*iso* and *trans*-10+11 C18:1 being appropriate predictors of CH₄ yield.

Restricting the selection of milk FA with a threshold concentration of 2.45 g/100 g of FA for reasonable accuracy using FTIR would have resulted in a best

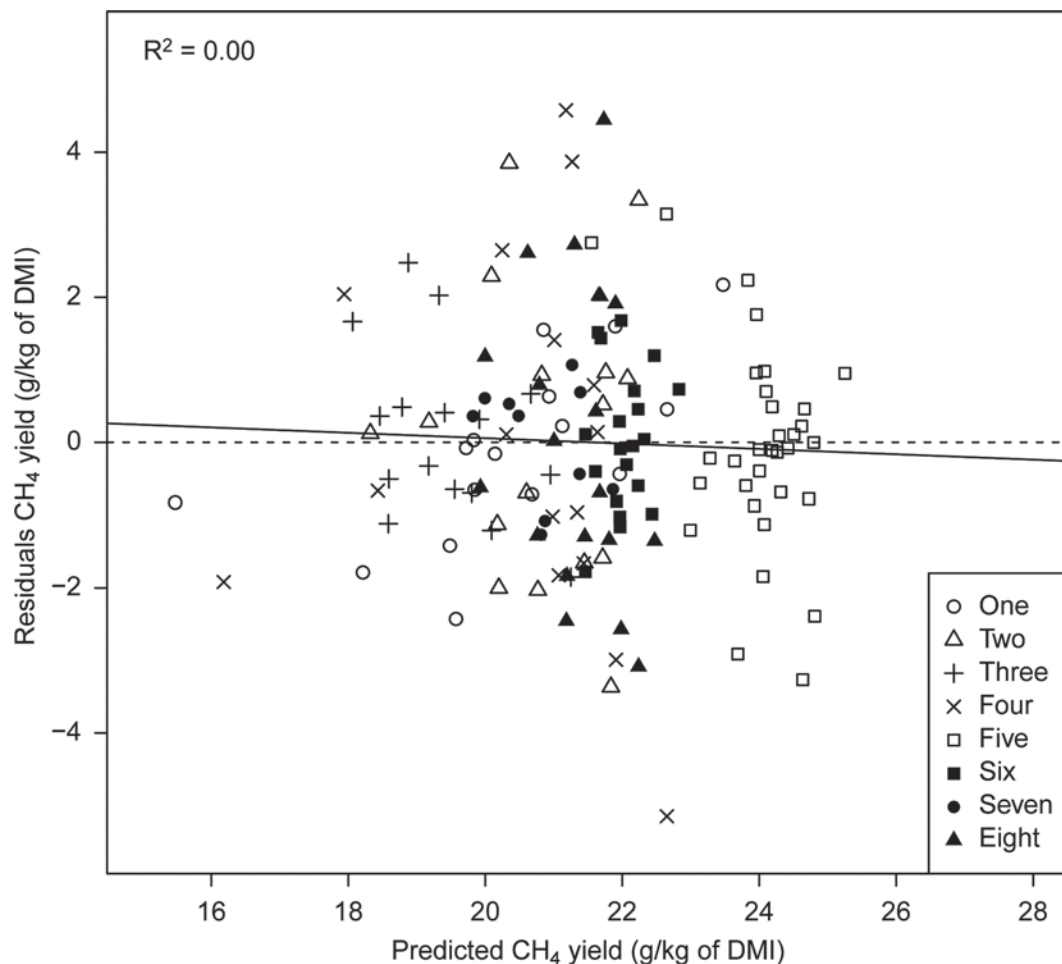


Figure 3. Residuals (predicted minus observed) and predicted CH₄ yield per unit of feed from Equation [3], including experiment as a discrete class variable with experiment effect not shown. The different symbols identify the 8 individual experiments.

prediction equation based on negative relationships of concentrations of C4:0 and *cis*-9 C18:1, and $R^2 = 0.28$. Including the FA concentrations mentioned by Soyeurt et al. (2011) in the selection procedure resulted in a best equation based on negative relationships of concentrations of C4:0, *cis*-9 C18:1, and total *trans*-C18:1, and $R^2 = 0.36$. It is noted that all FA available in studies 1 to 4 and 6 to 8 were included in the selection procedure because *trans*-10+11 C18:1 is the only *trans*-C18:1 fraction available in all 8 studies. Similar to prediction of CH₄ yield per unit of feed, these results indicate that current performance of FTIR limits the potential for predicting CH₄ yield per unit of milk based on milk FA profile, compared with gas chromatography.

The present meta-analysis showed that milk FA profile has moderate potential to predict CH₄ yield. Further improvement in ability of milk FA-based models to predict CH₄ yield may be achieved by distinguishing

diets with or without lipid supplements and by including diet composition characteristics. The present data set was too small to allow separate analyses of lipid-supplemented and non-lipid-supplemented diets, and more data may be required to analyze possible effects of lipid supplements on the relationship. On diet composition, Mohammed et al. (2011) already indicated that the combination of milk FA profile and diet characteristics may improve prediction performance. For practical application, this requires knowledge of diet composition (including forage to concentrate ratio and chemical composition of feed consumed), which may not always be available.

CONCLUSIONS

Various FA concentrations in milk fat appeared to be weakly, moderately, or strongly related to CH₄ yield per unit of feed. Milk C6:0, C8:0, C10:0, C16:0,

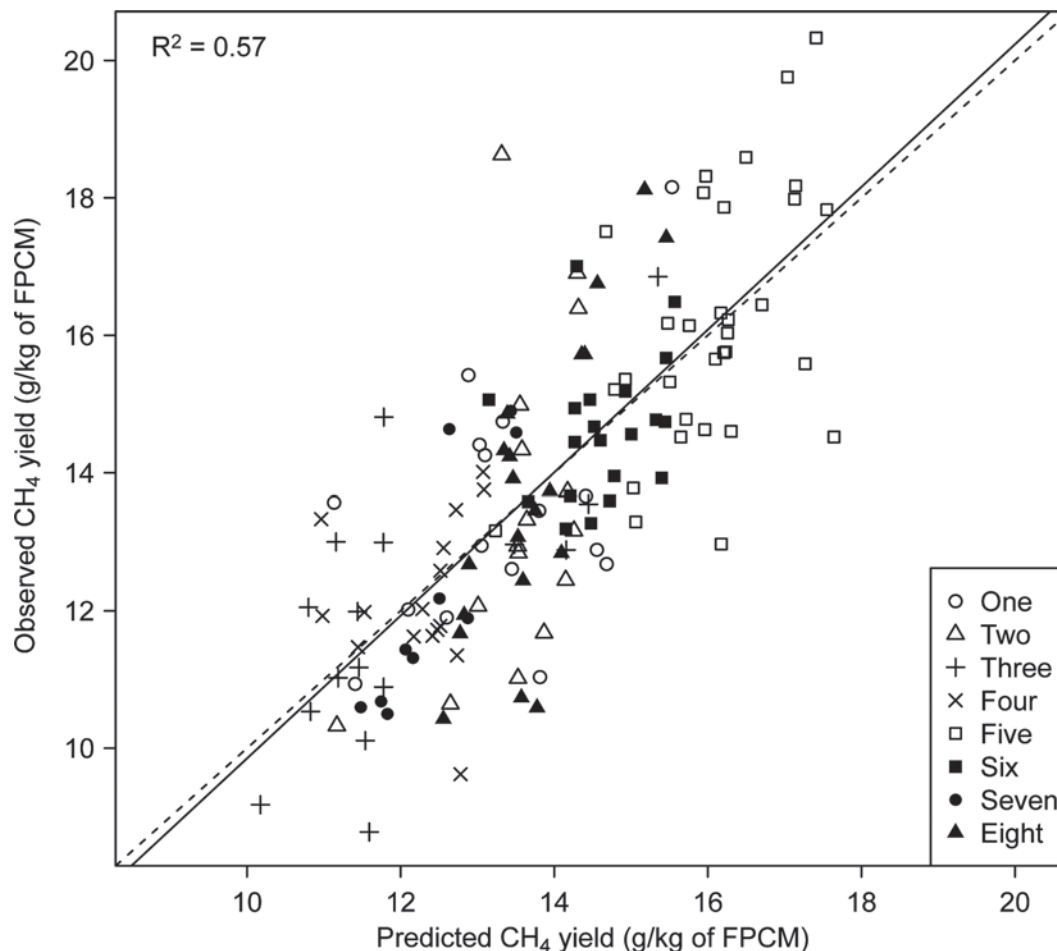


Figure 4. Observed versus predicted CH₄ yield per unit of milk (fat- and protein-corrected milk yield, FPCM) from Equation [4], including experiment as a discrete class variable with experiment effect not shown. The different symbols identify the 8 individual experiments. The line of unit slope represents the line of equivalence.

and C16:0-*iso* concentrations showed positive relationships, whereas milk *cis*-9,12 C18:2, *trans*-10+11 C18:1, *cis*-11 C18:1, *cis*-12 C18:1, *cis*-13 C18:1, and *trans*-16+*cis*-14 C18:1 concentrations showed weak to strong negative relationships with CH₄ yield. Milk OBCFA concentrations C14:0-*iso*, C15:0-*anteiso*, C15:0, and C17:0, as well as milk C4:0, C12:0, C14:0, *cis*-9 C14:1, C18:0, *cis*-9 C18:1, *cis*-9,12,15 C18:3, and C20:0 concentrations were not significantly related to CH₄ yield per unit of feed. When expressing CH₄ yield per unit of milk, relationships of milk C10:0, C15:0-*anteiso*, C16:0, C17:0, *trans*-10+11 C18:1, *cis*-11 C18:1, *cis*-9,12 C18:2, *cis*-9,12,15 C18:3, and C20:0 concentrations were in line with relationships expressing CH₄ yield per unit of feed. Concentrations of C18:1 isomers in milk fat showed more heterogeneity among the true correlation with CH₄ yield per unit of feed than concentrations of the saturated even-chain FA

C6:0, C8:0, C10:0, and C16:0. Mixed model multiple regression resulted in various milk FA included in optimal equations to predict CH₄ yield per unit of feed and per unit of milk, with C16:0-*iso* and *trans*-10+11 C18:1 concentrations appearing in both equations. These regressions indicated moderate potential for using milk FA profile to predict CH₄ yield per unit of feed and per unit of milk.

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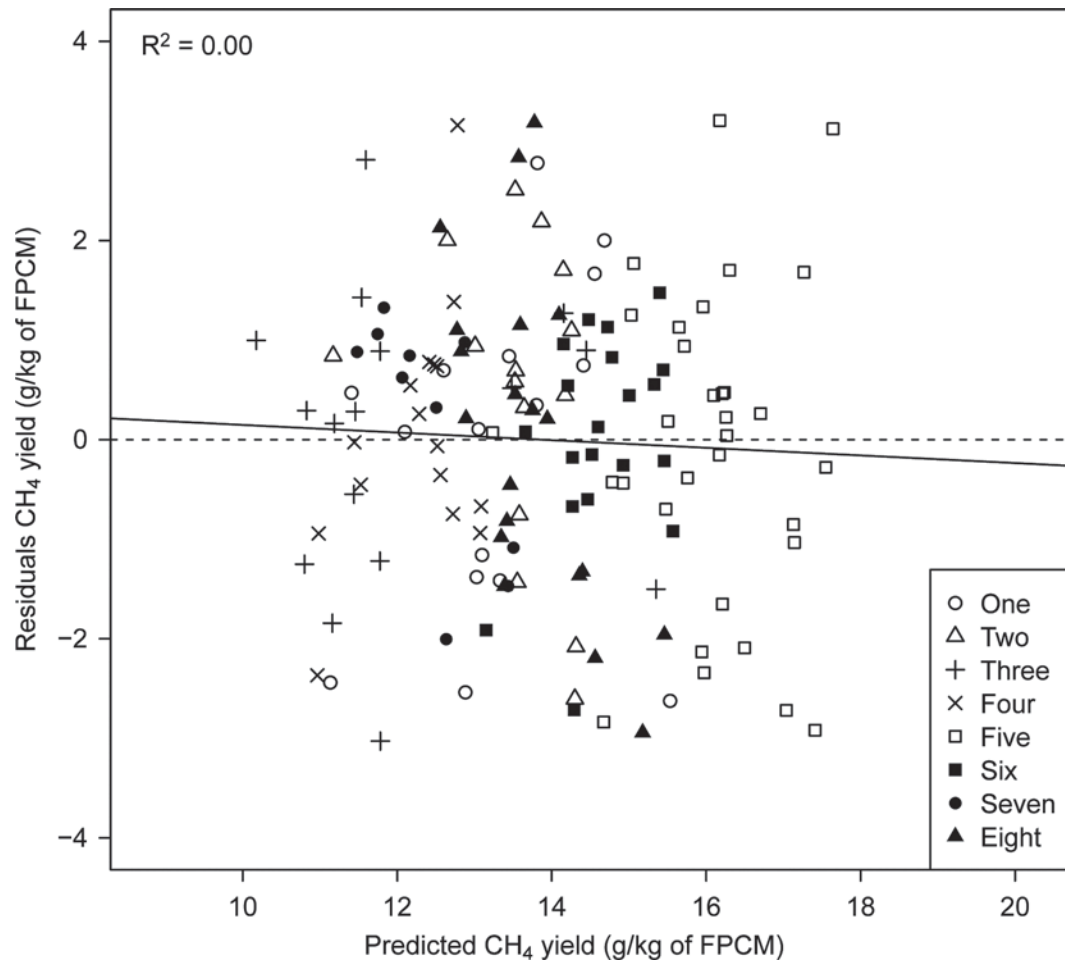


Figure 5. Residuals (predicted minus observed) and predicted CH_4 yield per unit of milk (fat- and protein-corrected milk yield, FPCM) from Equation [4], including experiment as a discrete class variable with experiment effect not shown. The different symbols identify the 8 individual experiments.

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