



Relationships between methane production and milk fatty acid profiles in dairy cattle

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ABSTRACT

There is a need to develop simple ways of quantifying and estimating CH₄ production in cattle. Our aim was to evaluate the relationship between CH₄ production and milk fatty acid (FA) profile in order to use milk FA profiles to predict CH₄ production in dairy cattle. Data from 3 experiments with dairy cattle with a total of 10 dietary treatments and 50 observations were used. Dietary treatments included supplementation with calcium fumarate, diallyldisulfide, caprylic acid, capric acid, lauric acid, myristic acid, extruded linseed, linseed oil and yucca powder. Methane was measured using open circuit indirect respiration calorimetry chambers and expressed as g/kg dry matter (DM) intake. Milk FA were analyzed by gas chromatography and individual FA expressed as a fraction of total FA. To determine relationships between milk FA profile and CH₄ production, univariate mixed model regression techniques were applied including a random experiment effect. A multivariate model was developed using a stepwise procedure with selection of FA based on the Schwarz Bayesian Information Criterion. Dry matter intake was 17.7 ± 1.83 kg/day, milk production was 27.0 ± 4.64 kg/day, and methane production was 21.5 ± 1.69 g/kg DM. Milk C8:0, C10:0, C11:0, C14:0 *iso*, C15:0 *iso*, C16:0 and C17:0 *anteiso* were positively related ($P < 0.05$) to CH₄ (g/kg DM intake), whereas C17:0 *iso*, *cis*-9 C17:1, *cis*-9 C18:1, *trans*-10+11 C18:1, *cis*-11 C18:1, *cis*-12 C18:1 and *cis*-14+*trans*-16 C18:1 were negatively related ($P < 0.05$) to CH₄. Multivariate analysis resulted in the equation: CH₄ (g/kg DM) = 24.6 ± 1.28 + 8.74 ± 3.581 × C17:0 *anteiso* – 1.97 ± 0.432 × *trans*-10+11 C18:1 – 9.09 ± 1.444 × *cis*-11 C18:1 + 5.07 ± 1.937 × *cis*-13 C18:1 (individual FA in g/100 g FA; $R^2 = 0.73$ after correction for experiment effect). This confirms the expected positive relationship between CH₄ and C14:0 *iso* and C15:0 *iso* in milk FA, as well as the negative relationship between CH₄ and various *trans*-intermediates, particularly *trans*-10+11 C18:1. However, in contrast with expectations, C15:0 and C17:0 were not related to CH₄ production. Milk FA profiles can predict CH₄ production in dairy cattle.

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Abbreviations: DM, dry matter; FA, fatty acid; OBCFA, odd- and branched-chain fatty acids; VFA, volatile fatty acids.

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1. Introduction

Various dietary strategies have been proposed to reduce production of CH₄ by dairy cattle (Beauchemin et al., 2009). Accurate measurements of CH₄ production from cattle in various dietary situations require complex and expensive techniques. Mathematical models may allow prediction of CH₄ production from cattle without extensive experiments. However, the accuracy of empirical models to predict CH₄ production for inventory or mitigation purposes is low (Ellis et al., 2010), and mechanistic models are complex and require inputs that are not commonly measured. Thus development of simple indicators to estimate CH₄ production in cattle is of substantive interest.

Vlaeminck and Fievez (2005) suggested that odd- and branched-chain fatty acids (OBCFA) in milk may be used as markers of microbial activity, as OBCFA have a strong relationship with molar proportions of individual volatile fatty acids (VFA) in the rumen (Vlaeminck et al., 2006), which in turn are related to CH₄ production (Ellis et al., 2008). In their model, Vlaeminck and Fievez (2005) reported a positive relationship of CH₄ predicted from rumen VFA molar proportions with C15:0 *iso*, and a negative relationship with C15:0 content of milk fat. However, in an experiment comparing a control diet with a myristic acid supplemented diet, Odongo et al. (2007) did not find reduced C15:0 *iso* or increased C15:0 at lower CH₄ production, although milk fat C14:0 *iso* was negatively related to CH₄ production. Chilliard et al. (2009) evaluated effects of various dietary linseed treatments on CH₄ production in dairy cattle and did find relationships of milk contents of C15:0 and C15:0 *iso* with CH₄, but relationships of other milk FA with CH₄ were stronger. Although milk FA profile may be a potential indicator of CH₄ production, actual determined relationships *in vivo* are limited to diets varying in type and availability of dietary FA. A wider variety of diets is required to explore the more general potential of milk FA profile as an indicator.

Our aim was to evaluate relationships between CH₄ production and milk FA profiles in dairy cattle, and to use FA profiles in milk to predict CH₄ production.

2. Materials and methods

2.1. Data

Data from three experiments, all designed as randomized block experiments, with a total of 50 observations from 100 cows were used. The experiments were completed in Wageningen and the Animal Care and Use Committee of Wageningen University, the Netherlands approved the experimental protocols. In all experiments, after an adaptation period of 12 days, cows were housed in pairs in two identical, open circuit, indirect climate respiration chambers for 6 (experiment 1) or 3 (experiment 2 and 3) days. Each pair of cows consisted of two cows on the same treatment, and consequently each observation is the mean of a pair of cows. Diets were fed as a total mixed ration twice daily and intake was restricted to 0.95 of the amount that was consumed voluntarily by the cow consuming the least within the pair of 2 (experiment 1) or 4 (experiments 2 and 3) cows. Cows were milked twice daily. In experiment 1 (Van Zijderveld et al., 2011a) 20 lactating Holstein–Friesian dairy cows were fed a control diet that included rumen inert fat from palm oil, or a diet supplemented with calcium fumarate in which the palm oil was substituted for lauric acid, myristic acid and linseed oil. The basal diet was (DM basis) 0.29 grass silage, 0.22 maize silage, 0.02 wheat straw and 0.47 concentrate. In experiment 2 (Van Zijderveld et al., 2011b) 40 lactating Holstein–Friesian dairy cows were fed a control diet or a diet containing diallyldisulfide, yucca plant powder, or calcium fumarate. The diet was 0.26 maize silage, 0.40 grass silage and 0.34 concentrates on a DM basis. In the third experiment (Van Zijderveld et al., 2011b), 40 lactating Holstein–Friesian dairy cows were fed a control diet or diets supplemented with extruded linseed, diallyldisulfide, or a mixture of caprylic acid and capric acid. The diet contained (DM basis) 0.41 grass silage, 0.35 maize silage and 0.24 concentrates.

Methane production was determined in 9 min intervals as described by Van Knegsel et al. (2007). Milk production was recorded during the presence of the cows in the respiration chambers and a sample was obtained at each milking. Samples were pooled, weighted by production, to one sample for analyses of milk composition. Milk FA composition of the cows per chamber was calculated as the weighted average of the respective analyzed FA composition and milk fat yield. After extraction and methylation, milk FA were analyzed by gas chromatography (Van Knegsel et al., 2007) and individual FA were expressed as a fraction of total FA. Peaks were identified using external standards (S37, Supelco, Bellefonte, PA, USA; OBCFA and various *trans*-FA, Larodan Fine Chemicals AB, Malmö, Sweden). The analysis did not allow several C18:1 isomers to be completely resolved and therefore some FA are summarized together in Table 1. The milk fat and protein contents were similar to contents of Dutch bovine milk (4.38 and 3.48 g/100 g milk; Heck et al., 2009).

2.2. Statistical analysis

To determine the relationship between individual milk FA and CH₄ production, mixed model univariate regression techniques (PROC MIXED of SAS, 2007) were applied which included a discrete random experiment effect and individual milk FA as fixed effects. Treating the experiment effect as a random effect caused the equation parameter estimates to be estimated first within study, and then averaged to obtain overall estimates. Distribution of random effects was assumed to be normal with an unstructured variance–covariance matrix for the intercepts and slopes. In addition, a multivariate model was developed using a stepwise procedure (PROC GLMSELECT of SAS) retaining the experiment effect in every step, with CH₄ production the independent variable and stepwise selection of FA based on the Schwarz Bayesian Information Criterion.

Table 1Summary statistics of experimental data used for modelling ($n=50$) [data from Van Zijderveld et al. (2011a,b)].

	Mean	SD	Minimum	Maximum
Dry matter intake (kg/day)	17.7	1.83	14.0	20.7
Milk production (kg/day)	27.0	4.64	17.6	35.1
Milk fat content (g/100 g milk)	4.36	0.643	3.23	6.24
Milk protein content (g/100 g milk)	3.30	0.287	2.86	3.99
Methane production (g/day)	381	51.7	279	456
Methane per kg feed (g/kg DM)	21.5	1.69	17.3	25.3
Milk fatty acids (g/100 g total fatty acids):				
C4:0	3.13	0.320	2.45	3.62
C6:0	2.09	0.241	1.42	2.44
C8:0	1.24	0.170	0.85	1.51
C10:0	2.83	0.502	1.86	3.75
C11:0	0.308	0.0570	0.181	0.414
C12:0	3.29	0.560	2.07	4.27
C13:0	0.123	0.0223	0.101	0.181
C14:0	11.87	2.131	8.60	18.24
C14:0 iso	0.153	0.0334	0.093	0.220
cis-9 C14:1	0.963	0.1967	0.566	1.55
C15:0	0.970	0.1482	0.715	1.270
C15:0 iso	0.245	0.0509	0.159	0.458
C15:0 anteiso	0.443	0.0615	0.328	0.573
C16:0	31.30	4.338	21.41	38.46
cis-9 C16:1	1.85	0.299	1.26	2.56
C17:0	0.584	0.1094	0.383	0.774
C17:0 iso	0.203	0.0755	0.113	0.374
C17:0 anteiso	0.227	0.0453	0.102	0.303
cis-9 C17:1	0.228	0.0534	0.121	0.385
C18:0	10.16	1.377	8.11	14.84
trans-6+7+8+9 C18:1	0.359	0.0722	0.249	0.543
trans-10+11 C18:1	1.10	0.411	0.506	2.32
trans-12 C18:1	0.305	0.1660	0.146	0.856
trans-13+14 C18:1	1.13	0.554	0.368	2.45
cis-9 C18:1	18.44	2.158	14.78	24.21
cis-11 C18:1	0.477	0.1029	0.304	0.756
cis-12 C18:1	0.237	0.1124	0.136	0.653
cis-13 C18:1	0.285	0.1181	0.110	0.651
cis-14+trans-16 C18:1	0.244	0.2104	0.104	0.903
cis-9,12 C18:2	1.30	0.244	0.569	1.82
cis-9, trans-11 C18:2	0.354	0.0938	0.175	0.627
trans-11, cis-15 C18:2	0.228	0.1798	0.100	0.771
cis-9,12,15 C18:3	0.547	0.1566	0.365	1.023
C20:0	0.129	0.0190	0.101	0.173

Adjusted independent variable values were calculated based on regression parameters of the final model to determine the r or R^2 corrected for experiment effect (St-Pierre, 2001).

3. Results and discussion

Dry matter intake is a major determinant of CH_4 production from cattle (e.g., Bannink et al., 2010). A higher DM intake will generally result in increased amounts of organic matter fermented in the rumen with associated production of VFA and gases. Indeed in our analysis, DM intake was positively related ($P<0.001$; $r=0.84$) to CH_4 production with a slope of 23.1 ± 2.38 g CH_4 /kg DM intake. To evaluate dietary mitigations, variation in the amount of CH_4 produced/unit feed is of more interest than total output of CH_4 because it avoids confounding effects of DM intake on CH_4 production, and because DM intake is known or can be estimated with reasonable accuracy in stall fed cows. Therefore, CH_4 produced/kg feed DM was related to individual FA concentrations in milk fat (Table 2).

Consistent with Odongo et al. (2007) and Chilliard et al. (2009), CH_4 production was positively correlated ($P<0.05$) with C8:0, C10:0, C11:0 and C16:0 (all g/100 g total FA). However, Johnson et al. (2002) did report reduced concentrations of C10:0, C12:0, C14:0 and C16:0 in milk fat upon supplementation with cottonseed and canola seed, and CH_4 production was not affected. These FA are mainly derived from *de novo* synthesis in the mammary gland from acetate and 3-hydroxy butyrate (Bernard et al., 2008). Formation of acetate in the rumen, largely as the result of fermentation of fibre (Bannink et al., 2008), results in the production of hydrogen gas that is used to produce CH_4 by methanogenic archaea. A range of dietary unsaturated FA may reduce CH_4 production (Beauchemin et al., 2009). Since various unsaturated FA are also known to inhibit *de novo* synthesis of FA with 16 C or less, with the possible exception of C4:0 (Bernard et al., 2008), this may also explain the relationship between CH_4 and *de novo* synthesised FA. Indeed, of FA with 16 C or less, only C4:0 tended ($P=0.07$) to be negatively related to CH_4 production.

Table 2

Linear regression between methane production (g/kg feed DM) and milk fatty acid concentration (g/100 g total fatty acids) with experiment included as random effect.

	Intercept	SE	Slope	SE	Slope P	r
C4:0	25.8	2.40	-1.40	0.759	0.07	-0.27
C6:0	18.7	2.18	1.31	1.039	0.21	0.19
C8:0	17.5	1.71	3.17	1.361	0.02	0.32
C10:0	18.6	1.33	1.02	0.463	0.03	0.30
C11:0	17.6	1.21	12.5	3.88	0.002	0.42
C12:0	19.4	1.42	0.641	0.4255	0.14	0.21
C13:0	22.3	1.78	-5.92	13.902	0.67	-0.10
C14:0	23.2	1.43	-0.151	0.1158	0.20	-0.20
C14:0 <i>iso</i>	18.7	1.26	19.5	8.04	0.02	0.37
<i>cis</i> -9 C14:1	22.0	1.23	-0.593	1.2279	0.63	-0.07
C15:0	19.3	1.58	2.23	1.613	0.17	0.20
C15:0 <i>iso</i>	18.1	1.09	13.8	4.36	0.003	0.42
C15:0 <i>anteiso</i>	21.7	1.99	-0.676	4.43	0.88	-0.03
C16:0	17.4	1.68	0.130	0.0531	0.02	0.34
<i>cis</i> -9 C16:1	21.0	1.53	0.232	0.8110	0.78	0.04
C17:0	19.1	1.28	4.04	2.151	0.07	0.26
C17:0 <i>iso</i>	23.1	0.80	-8.18	3.494	0.02	-0.37
C17:0 <i>anteiso</i>	17.5	1.10	17.5	4.78	<0.001	0.47
<i>cis</i> -9 C17:1	25.1	1.20	-17.5	4.41	<0.001	-0.55
C18:0	21.5	1.82	-0.010	0.1759	0.96	-0.01
<i>trans</i> -6+7+8+9 C18:1	23.5	1.20	-5.74	3.274	0.09	-0.25
<i>trans</i> -10+11 C18:1	23.5	0.64	-1.86	0.537	0.001	-0.46
<i>trans</i> -12 C18:1	22.2	0.50	-2.58	1.425	0.08	-0.25
<i>trans</i> -13+14 C18:1	21.9	0.67	-0.451	0.4805	0.35	-0.15
<i>cis</i> -9 C18:1	26.2	2.08	-0.257	0.1120	0.03	-0.33
<i>cis</i> -11 C18:1	26.0	1.09	-9.80	1.957	<0.001	-0.61
<i>cis</i> -12 C18:1	22.7	0.55	-5.04	2.081	0.02	-0.34
<i>cis</i> -13 C18:1	20.2	0.70	4.36	2.247	0.06	0.31
<i>cis</i> -14+ <i>trans</i> -16 C18:1	22.1	0.42	-2.57	1.207	0.04	-0.33
<i>cis</i> -9,12 C18:2	24.3	1.84	-2.20	1.332	0.11	-0.32
<i>cis</i> -9, <i>trans</i> -11 C18:2	23.2	0.93	-5.02	2.509	0.05	-0.28
<i>trans</i> -11, <i>cis</i> -15 C18:2	22.0	0.44	-2.94	1.524	0.06	-0.29
<i>cis</i> -9,12,15 C18:3	21.3	0.92	0.269	1.5774	0.87	0.03
C20:0	22.0	2.15	-6.36	16.37	0.70	-0.08

Consistent with theoretical expectations (Vlaeminck and Fievez, 2005), and experimental data (Chilliard et al., 2009), C14:0 *iso* and C15:0 *iso* in milk fat were positively related ($P=0.02$ and 0.003 , respectively) to CH_4 , but C17:0 *iso* was negatively related ($P=0.02$). Fibrolytic bacteria are enriched in C14:0 *iso* and C15:0 *iso*, and an increase in dietary forage to concentrate ratio, which will generally increase CH_4 production, is also associated with higher levels of C14:0 *iso* and C15:0 *iso* in milk fat (Vlaeminck et al., 2006). Odongo et al. (2007) reported a numerical decrease of C17:0 *anteiso* accompanied by a decrease of CH_4 in the myristic supplemented diet. In our study, a positive relationship ($P<0.001$) between CH_4 and C17:0 *anteiso* also occurred. Cabrita et al. (2003) reported a negative relationship between dietary crude protein content and C17:0 *anteiso* content in milk fat, and a positive relationship between dietary fibre content and C17:0 *anteiso*. Because, stoichiometrically, fermentation of protein is associated with lower CH_4 production compared with fermentation of fibre or sugars (Bannink et al., 2008), such associations between dietary crude protein, fibre and milk C17:0 *anteiso* may explain the positive relationship of this FA with CH_4 .

A high propionic acid level in the rumen is associated with low CH_4 production, and propionic acid is a substrate for *de novo* synthesis of C15:0 and C17:0. Thus Vlaeminck and Fievez (2005) expected a negative relationship between these odd chain FA and CH_4 , but Chilliard et al. (2009) reported a positive correlation between these odd chain FA and CH_4 . Odongo et al. (2007) did not find changes in C15:0 and C17:0 contents with changes in CH_4 production. In our analysis, C15:0 was not related with CH_4 and C17:0 tended ($P=0.07$) to be positively related. However, *cis*-9 C17:1 was negatively related ($P<0.001$) to CH_4 . *Cis*-9 C17:1 is a desaturation product of C17:0 in the mammary gland. The sum of C17:0 and *cis*-9 C17:1 was negatively related ($P=0.03$) to CH_4 production (results not shown). Supplementation with linseed changed mammary desaturation activity, which may have caused relationships between milk FA and CH_4 in Chilliard et al. (2009) to differ from others, and in our findings.

Milk content of many unsaturated FA, such as *cis*-9 C18:1, *trans*-10+11 C18:1, *cis*-11 C18:1, *cis*-12 C18:1 and *cis*-14+*trans*-16 C18:1, were all negatively associated with CH_4 production, which largely agrees with Chilliard et al. (2009). However, In Odongo et al. (2007), supplementation with myristic acid decreased CH_4 production but *trans*-10 C18:1, *trans*-11 C18:1, and *cis*-11 C18:1 were not affected, whilst *cis*-9 C18:1 and *cis*-12 C18:1 were lower in the supplemented diet. A number of these unsaturated FA originate in the rumen, but the microorganisms and enzymes responsible for their production are not yet well characterized or understood (Wallace et al., 2007).

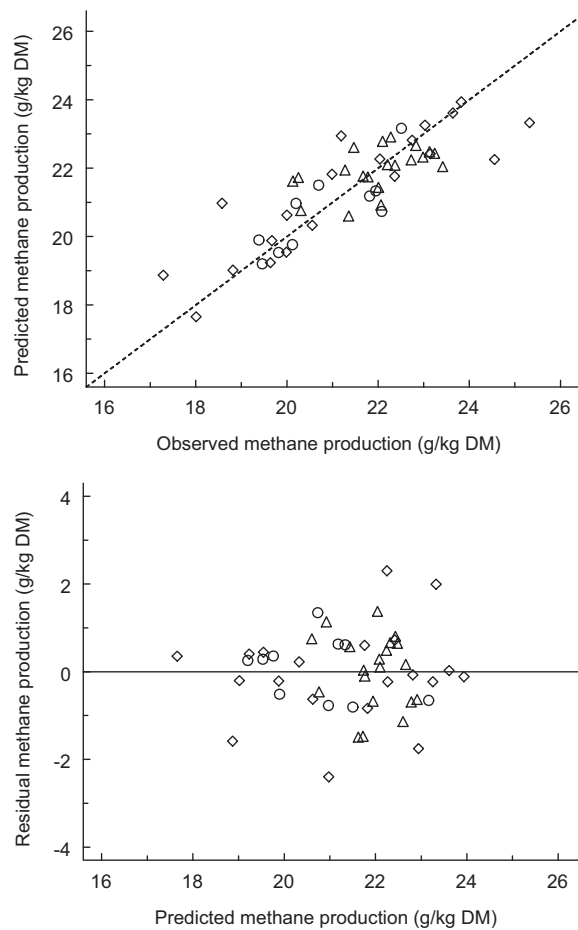


Fig. 1. Observed and predicted CH_4 production, and residuals (*i.e.*, observed – predicted) CH_4 production, from the multivariate analysis including experiment as a discrete class variable. Predicted CH_4 (g/kg DM) = $24.6 + 8.74 \times \text{C17:0 anteiso} - 1.97 \times \text{trans-10+11 C18:1} - 9.09 \times \text{cis-11 C18:1} + 5.07 \times \text{cis-13 C18:1}$ (individual FA in g/100 g of total FA; $R^2 = 0.73$ after correction for experiment effect (St-Pierre, 2001) with experiment effect not shown). Δ , experiment 1; \circ , experiment 2; \diamond , experiment 3. The line of unit slope (dotted line) represents the line of equivalence.

Supplementation with various dietary fat sources may reduce CH_4 production (Beauchemin et al., 2009) and increase formation of ruminal biohydrogenation intermediates (Harfoot and Hazlewood, 1997). Fibre degradation in the rumen may decrease with dietary addition of fat, and this further explains the variation in the relationships between contents of various biohydrogenation intermediates and CH_4 production.

Multivariate analysis using a stepwise approach resulted in the equation (experiment effect not presented):

$$\text{CH}_4 \text{ (g/kg DM)} = 24.6 \pm 1.28 + 8.74 \pm 3.581 \times \text{C17:0 anteiso} - 1.97 \pm 0.432 \times \text{trans} \\ -10 + 11 \text{ C18:1} - 9.09 \pm 1.444 \times \text{cis-11 C18:1} + 5.07 \pm 1.937 \times \text{cis-13 C18:1}$$

where individual FA are in g/100 g FA and $R^2 = 0.73$ after correction for the experiment effect (St-Pierre, 2001) with all parameters $P < 0.02$ (see Fig. 1 for observed and predicted relationship and residual CH_4 production). The R^2 of this equation is lower than the best equation derived by Chilliard et al. (2009). However, Chilliard et al. (2009) obtained relationships using absolute CH_4 production (g/day) rather than CH_4 produced/kg feed DM, and they only used diets that varied in supply and availability of linolenic acid, which may have increased the R^2 compared with our approach.

However our study shows high potential for milk FA to be used as an indicator of CH_4 produced/kg feed consumed. The number of data ($n = 50$) and studies ($n = 3$) used in our analysis were limited and, within experiment, there was no variation in type, composition or proportion of dietary forage and concentrate, which may limit application of our equation to other diets. For example, the high contents of *trans*-10+11 C18:1 (10 g/100 g milk total FA) by feeding docosahexaenoic acid enriched diets (Boeckaert et al., 2008) would likely result in predicted CH_4 production being close to zero. More data are needed to confirm relationships between milk FA profile and CH_4 production for a wide range of dietary conditions.

4. Conclusions

Various milk fatty acids showed moderate relationships with CH₄ production in dairy cattle. In particular, C14:0 *iso*, C15:0 *iso* and C17:0 *anteiso* were positively related with CH₄ production, and *cis*-9 C17:1 and various FA arising from ruminal biohydrogenation of FA were negatively related with CH₄ production. Milk FA profile can be used to predict the formation of CH₄ in dairy cattle, but more data for a wide range of diets are required to confirm this prediction.

Conflict of interest statement

None.

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