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

Dijkstra, J.; Zijderveld, S.M. van; Apajalahti, J.A.; Bannink, A.; Gerrits, W.J.J.; Newbold, J.R.; Perdok, H.B.; Berends, H.

Dijkstra, J.; Zijderveld, S.M. van; Apajalahti, J.A.; Bannink, A.; Gerrits, W.J.J.; Newbold, J.R.; Perdok, H.B.; Berends, H. (2011) Relationships between methane production and milk fatty acid profiles in dairy cattle. Animal Feed Science and Technology 166-167 (2011). - ISSN 0377-8401 - p. 590 - 595..

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

J. Dijkstra[a*], S.M. van Zijderveld[b], J.A. Apajalahti[c], A. Bannink[d], W.J.J. Gerrits[a], J.R. Newbold[b], H.B. Perdok[b], H. Berends[a]

[a] *Animal Nutrition Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands*
[b] *Provimi Research Centre, Veilingweg 23, 5334 LD Velddriel, the Netherlands*
[c] *Alimetrics, Koskelontie 19B, FI-02920 Espoo, Finland*
[d] *Livestock Research, Animal Sciences Group, Wageningen University and Research Centre, Lelystad, the Netherlands*

*Corresponding author:
phone: +31 317 484082
fax: +31 317 484062
e-mail: jan.dijkstra@wur.nl

Abstract

There is a need to develop simple ways of quantifying and estimating methane production in cattle. Our aim was to evaluate the relationship between methane production and milk fatty acid (FA) profile in order to use milk FA profiles to predict methane production in dairy cattle. Data from three 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 methane 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 methane (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 methane. Multivariate analysis resulted in the equation: methane (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 methane and C14:0 *iso* and C15:0 *iso* in milk FA, as well as the negative relationship between methane and various *trans*-intermediates, particularly *trans*-10+11 C18:1. However, in contrast with expectations, C15:0 and C17:0 were not related to methane production. Milk FA profiles can predict methane production in dairy cattle.

This paper is part of the special issue entitled: Greenhouse Gases in Animal Agriculture – Finding a Balance between Food and Emissions, Guest Edited by T.A. McAllister, Section Guest Editors; K.A. Beauchemin, X. Hao, S. McGinn and Editor for Animal Feed Science and Technology, P.H. Robinson.

Keywords: methane, dairy cow, milk fatty acid profile

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

1. Introduction

Various dietary strategies have been proposed to reduce production of methane by dairy cattle (Beauchemin et al., 2009). Accurate measurements of methane production from cattle in various dietary situations require complex and expensive techniques. Mathematical models may allow prediction of methane production from cattle without undertaking extensive experiments. However, the accuracy of empirical models to predict methane 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 methane 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 methane production (Ellis et al., 2008). In their model, Vlaeminck and Fievez (2005) reported a positive relationship of methane 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 methane production, although milk fat C14:0 *iso* was negatively related to methane production. Chilliard et al. (2009) evaluated effects of various dietary linseed treatments on methane production in dairy cattle and did find relationships of milk contents of C15:0 and C15:0 *iso* with methane, but relationships of other milk FA with methane were stronger. Although milk FA profile may be a potential indicator of methane 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 methane production and milk FA profiles in dairy cattle, and to use FA profiles in milk to predict methane production.

2. Material 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 (experiments 2 and 3) days. Each pair of cows consisted of two cows on the same treatment, and consequently each observation is the mean value 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. The 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 milk protein contents were similar to average 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 methane production, a 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, 2007) retaining the experiment effect in every step, with methane production being the independent variable and stepwise selection of FA based on the Schwarz Bayesian Information Criterion. 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 methane 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 the present analysis, DM intake was positively related ($P<0.001$; r = 0.84) to methane production with a slope of 23.1 ± 2.38 g methane/kg DM intake. To evaluate dietary mitigation options, variation in the amount of methane produced per unit feed is of more interest than total output of methane because it avoids confounding effects of DM intake on methane production, and because DM intake is known or can be estimated with reasonable accuracy in stall-fed cows. Therefore, methane produced per kg of feed DM was related to individual FA concentrations in milk fat, and results are in Table 2.

Consistent with Odongo et al. (2007) and Chilliard et al. (2009), methane 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 methane 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 methane by methanogenic archaea. A range of dietary unsaturated FA may reduce methane production (Beauchemin et al., 2009). Since various unsaturated FA are also known to inhibit *de novo* synthesis of FA with 16 carbons or less, with the possible exception of C4:0 (Bernard et al., 2008), this may also explain the relationship between methane and *de novo* synthesised FA. Indeed, of FA with 16 carbons or less, only C4:0 tended (P=0.07) to be negatively related to methane production.

Consistent with theoretical expectations (Vlaeminck and Fievez, 2005), and with 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 methane, 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 methane 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 methane in the myristic supplemented diet. In our study, a positive relationship (P<0.001) between methane 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 a lower methane 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 methane.

A high propionic acid level in the rumen is associated with low methane 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 methane, but Chilliard et al. (2009) reported a positive correlation between these odd chain FA and methane. Odongo et al. (2007) did not find changes in C15:0 and C17:0 contents with changes in methane production. In our analysis, C15:0 was not related with methane and C17:0 tended (P=0.07) to be positively related. However, *cis*-9 C17:1 was negatively related (P<0.001) to methane. *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 methane production (results not shown). Supplementation with linseed changed mammary desaturation activity, which may have caused relationships between milk FA and methane 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 methane production, which largely agrees with Chilliard et al. (2009). However, In Odongo et al. (2007), supplementation with myristic acid decreased methane 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).

Supplementation with various dietary fat sources may reduce methane 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 methane production.

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

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

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 Figure 1 for observed and predicted relationship and residual methane 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 methane production (g/day) rather than methane 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 methane 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 methane production being close to zero. More data are needed to confirm relationships between milk FA profile and methane production for a wide range of dietary conditions.

4. Conclusions

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

Acknowledgements

Data on DM intake, milk production and methane production were obtained in experiments partially funded by SenterNovem, an agency of the Dutch Ministry of Economic Affairs.

References

Bannink, A., Smits, M.C.J., Kebreab, E., Mills, J.A.N., Ellis, J.L., Klop, A., France, J., Dijkstra, J., 2010. Simulating the effects of grassland management and grass ensiling on methane emission from lactating cows. J. Agric. Sci., Camb. 148, 55-72.

Bannink, A., France, J., Lopez, S., Gerrits, W.J.J., Kebreab, E., Tamminga, S., Dijkstra, J., 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Anim. Feed Sci. Technol. 143, 3-26.

Beauchemin, K.A., McAllister, T.A., McGinn, S.M., 2009. Dietary mitigation of enteric methane from cattle. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 4, No. 035.

Bernard, L., Leroux, C., Chilliard, Y., 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. Adv. Exp. Med. Biol. 606, 67-108.

Boeckaert, C., Vlaeminck, B., Dijkstra, J., Issa-Zacharia, A., van Nespen, T., Van Straalen, W., Fievez, V., 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J. Dairy Sci. 91, 4714-4727.

Cabrita, A.R.J., Fonseca, A.J.M., Dewhurst, R.J., Gomes, E., 2003. Nitrogen supplementation of corn silages. 2. Assessing rumen function using fatty acid profiles of bovine milk. J. Dairy Sci. 86, 4020–4032.

Chilliard, Y., Martin, C., Rouel, J., Doreau, M., 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J. Dairy Sci. 92, 5199-5211.

Ellis, J.L., Bannink, A., France, J., Kebreab, E., Dijkstra, J., 2010. Evaluation of enteric methane prediction equations for dairy cows used in whole farm models. Glob. Change Biol. 16, 3246-3256.

Ellis, J.L., Dijkstra, J., Kebreab, E., Bannink, A., Odongo, N.E., McBride, B.W., France, J., 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agric. Sci., Camb. 146, 213-233.

Harfoot G.C., Hazlewood, G.P., 1997. Lipid metabolism in the rumen, in: Hobson, P.N., C.S. Stewart (Eds.), The Rumen Microbial Ecosystem. 2nd ed. Blackie Academic & Professional, London, UK, pp 382-426.

Heck, J.M.L., van Valenberg, H.J.F., Dijkstra, J., van Hooijdonk, A.C.M., 2009. Seasonal variation in the Dutch bovine raw milk composition. J. Dairy Sci. 92, 4745-4755.

Johnson, K.A., Kincaid, R.L., Westberg, H.H., Gaskins, C.T., Lamb, B.K., Cronrath, J.D., 2002. The effect of oilseeds in diets of lactating cows on milk production and methane emissions. J. Dairy Sci. 85, 1509-1515.

Odongo, N.E., Or-Rashid, M.M., Kebreab, E., France, J., McBride, B.W., 2007. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. J. Dairy Sci. 90, 1851-1858.

SAS Institute Inc. (2007) SAS/STAT® User’s Guide, Version 9.2. Cary, NC, USA.

St-Pierre, N.R., 2001. Invited review: integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84, 741-755.

Van Knegsel, A.T.M., Van den Brand, H., Dijkstra, J., Van Straalen, W.M., Heetkamp, M.J.W., Tamminga, S., Kemp, B., 2007. Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. J. Dairy Sci. 90, 1467-1476.

Van Zijderveld, S.M., Fonken, B.C.J., Dijkstra, J., Gerrits,W.J.J., Perdok, H.B., Fokkink, W.B., Newbold, J.R., 2011a. Effects of a combination of feed additives on methane production, diet digestibility and animal performance in lactating dairy cows. J. Dairy Sci. 94, 1445–1454.

Van Zijderveld, S.M., Dijkstra, J., Perdok, H.B., Newbold, J.R., Gerrits, W.J.J., 2011b. Dietary inclusion of diallyldisulfide, yuccapowder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows. J. Dairy Sci 94, 3094-3104.

Vlaeminck, B., Fievez, V., 2005. Milk odd and branched-chain fatty acids to predict ruminal methanogenesis in dairy cows. Comm. Appl. Biol. Sci., Ghent Univ. 70, 43-47.

Vlaeminck, B., Fievez, V., Tamminga, S., Dewhurst, R.J., Van Vuuren, A.M., De Brabander, D., Demeyer, D., 2006. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern. J. Dairy Sci. 89, 3954–3964.

Wallace, R.J., McKain, N., Shingfield, K.J., Devillard, E., 2007. Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. J. Lipid Res. 48, 2247–2254.

Table 1

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

	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

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 *iso*	18.7	1.26	19.5	8.04	0.02	0.37
cis-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 *iso*	18.1	1.09	13.8	4.36	0.003	0.42
C15:0 *anteiso*	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
cis-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 *iso*	23.1	0.80	-8.18	3.494	0.02	-0.37
C17:0 *anteiso*	17.5	1.10	17.5	4.78	<0.001	0.47
cis-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
trans-6+7+8+9 C18:1	23.5	1.20	-5.74	3.274	0.09	-0.25
trans-10+11 C18:1	23.5	0.64	-1.86	0.537	0.001	-0.46
trans-12 C18:1	22.2	0.50	-2.58	1.425	0.08	-0.25
trans-13+14 C18:1	21.9	0.67	-0.451	0.4805	0.35	-0.15
cis-9 C18:1	26.2	2.08	-0.257	0.1120	0.03	-0.33
cis-11 C18:1	26.0	1.09	-9.80	1.957	<0.001	-0.61
cis-12 C18:1	22.7	0.55	-5.04	2.081	0.02	-0.34
cis-13 C18:1	20.2	0.70	4.36	2.247	0.06	0.31
cis-14+*trans*-16 C18:1	22.1	0.42	-2.57	1.207	0.04	-0.33
cis-9,12 C18:2	24.3	1.84	-2.20	1.332	0.11	-0.32
cis-9, *trans*-11 C18:2	23.2	0.93	-5.02	2.509	0.05	-0.28
trans-11, *cis*-15 C18:2	22.0	0.44	-2.94	1.524	0.06	-0.29
cis-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

Figure 1. Observed and predicted methane production, and residuals (*i.e.*, observed – predicted) methane production, from the multivariate analysis including experiment as a discrete class variable. Predicted methane (g/kg DM) = 24.6 + 8.74 × C17:0 *anteiso* – 1.97 × *trans*-10+11 C18:1 – 9.09 × *cis*-11 C18:1 + 5.07 × *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; ○, experiment 2; ◊, experiment 3. The line of unit slope (dotted line) represents the line of equivalence.