



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

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

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30 Abstract

31 There is a need to develop simple ways of quantifying and estimating methane
32 production in cattle. Our aim was to evaluate the relationship between methane
33 production and milk fatty acid (FA) profile in order to use milk FA profiles to predict
34 methane production in dairy cattle. Data from three experiments with dairy cattle with
35 a total of 10 dietary treatments and 50 observations were used. Dietary treatments
36 included supplementation with calcium fumarate, diallyldisulfide, caprylic acid,
37 capric acid, lauric acid, myristic acid, extruded linseed, linseed oil and yucca powder.
38 Methane was measured using open-circuit indirect respiration calorimetry chambers
39 and expressed as g/kg dry matter (DM) intake. Milk FA were analyzed by gas
40 chromatography and individual FA expressed as a fraction of total FA. To determine
41 relationships between milk FA profile and methane production, univariate mixed
42 model regression techniques were applied including a random experiment effect. A
43 multivariate model was developed using a stepwise procedure with selection of FA
44 based on the Schwarz Bayesian Information Criterion. Dry matter intake was $17.7 \pm$
45 1.83 kg/day, milk production was 27.0 ± 4.64 kg/day, and methane production was
46 21.5 ± 1.69 g/kg DM. Milk C8:0, C10:0, C11:0, C14:0 *iso*, C15:0 *iso*, C16:0 and
47 C17:0 *anteiso* were positively related ($P < 0.05$) to methane (g/kg DM intake), whereas
48 C17:0 *iso*, *cis*-9 C17:1, *cis*-9 C18:1, *trans*-10+11 C18:1, *cis*-11 C18:1, *cis*-12 C18:1
49 and *cis*-14+*trans*-16 C18:1 were negatively related ($P < 0.05$) to methane. Multivariate
50 analysis resulted in the equation: methane (g/kg DM) = $24.6 \pm 1.28 + 8.74 \pm 3.581 \times$
51 C17:0 *anteiso* – $1.97 \pm 0.432 \times$ *trans*-10+11 C18:1 – $9.09 \pm 1.444 \times$ *cis*-11 C18:1 +
52 $5.07 \pm 1.937 \times$ *cis*-13 C18:1 (individual FA in g/100 g FA; $R^2 = 0.73$ after correction
53 for experiment effect). This confirms the expected positive relationship between
54 methane and C14:0 *iso* and C15:0 *iso* in milk FA, as well as the negative relationship
55 between methane and various *trans*-intermediates, particularly *trans*-10+11 C18:1.
56 However, in contrast with expectations, C15:0 and C17:0 were not related to methane
57 production. Milk FA profiles can predict methane production in dairy cattle.

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62 **Keywords:** methane, dairy cow, milk fatty acid profile

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

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67 **1. Introduction**

68 Various dietary strategies have been proposed to reduce production of methane by
69 dairy cattle (Beauchemin et al., 2009). Accurate measurements of methane production
70 from cattle in various dietary situations require complex and expensive techniques.
71 Mathematical models may allow prediction of methane production from cattle without
72 undertaking extensive experiments. However, the accuracy of empirical models to
73 predict methane production for inventory or mitigation purposes is low (Ellis et al.,
74 2010), and mechanistic models are complex and require inputs that are not commonly
75 measured. Thus development of simple indicators to estimate methane production in
76 cattle is of substantive interest.

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

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

96

97 **2. Material and methods**

98 *2.1. Data*

99 Data from three experiments, all designed as randomized block experiments, with
100 a total of 50 observations from 100 cows were used. The experiments were completed
101 in Wageningen and the Animal Care and Use Committee of Wageningen University,
102 the Netherlands, approved the experimental protocols. In all experiments, after an
103 adaptation period of 12 days, cows were housed in pairs in two identical, open-circuit,
104 indirect climate respiration chambers for 6 (experiment 1) or 3 (experiments 2 and 3)
105 days. Each pair of cows consisted of two cows on the same treatment, and
106 consequently each observation is the mean value of a pair of cows. Diets were fed as a
107 total mixed ration twice daily and intake was restricted to 0.95 of the amount that was
108 consumed voluntarily by the cow consuming the least within the pair of 2 (experiment
109 1) or 4 (experiments 2 and 3) cows. Cows were milked twice daily. In experiment 1
110 (Van Zijderveld et al., 2011a) 20 lactating Holstein-Friesian dairy cows were fed a
111 control diet that included rumen inert fat from palm oil, or a diet supplemented with
112 calcium fumarate in which the palm oil was substituted for lauric acid, myristic acid
113 and linseed oil. The basal diet was (DM basis) 0.29 grass silage, 0.22 maize silage,
114 0.02 wheat straw and 0.47 concentrate. In experiment 2 (Van Zijderveld et al., 2011b)
115 40 lactating Holstein-Friesian dairy cows were fed a control diet or a diet containing
116 diallyldisulfide, yucca plant powder, or calcium fumarate. The diet was 0.26 maize
117 silage, 0.40 grass silage and 0.34 concentrates on a DM basis. In the third experiment
118 (Van Zijderveld et al., 2011b), 40 lactating Holstein-Friesian dairy cows were fed a
119 control diet or diets supplemented with extruded linseed, diallyldisulfide, or a mixture
120 of caprylic acid and capric acid. The diet contained (DM basis) 0.41 grass silage, 0.35
121 maize silage and 0.24 concentrates.

122 Methane production was determined in 9 min intervals as described by Van
123 Knegsel et al. (2007). Milk production was recorded during the presence of the cows
124 in the respiration chambers and a sample was obtained at each milking. The samples
125 were pooled, weighted by production, to one sample for analyses of milk
126 composition. Milk FA composition of the cows per chamber was calculated as the
127 weighted average of the respective analyzed FA composition and milk fat yield. After
128 extraction and methylation, milk FA were analyzed by gas chromatography (Van
129 Knegsel et al. 2007) and individual FA were expressed as a fraction of total FA. Peaks
130 were identified using external standards (S37, Supelco, Bellefonte, PA, USA; OBCFA

131 and various *trans*-FA, Larodan Fine Chemicals AB, Malmö, Sweden). The analysis
132 did not allow several C18:1 isomers to be completely resolved and therefore some FA
133 are summarized together in Table 1. The milk fat and milk protein contents were
134 similar to average contents of Dutch bovine milk (4.38 and 3.48 g/100 g milk; Heck et
135 al., 2009).

136 2.2. Statistical analysis

137 To determine the relationship between individual milk FA and methane
138 production, a mixed model univariate regression techniques (PROC MIXED of SAS,
139 2007) were applied which included a discrete random experiment effect and
140 individual milk FA as fixed effects. Treating the experiment effect as a random effect
141 caused the equation parameter estimates to be estimated first within study, and then
142 averaged to obtain overall estimates. Distribution of random effects was assumed to
143 be normal with an unstructured variance-covariance matrix for the intercepts and
144 slopes. In addition, a multivariate model was developed using a stepwise procedure
145 (PROC GLMSELECT of SAS, 2007) retaining the experiment effect in every step,
146 with methane production being the independent variable and stepwise selection of FA
147 based on the Schwarz Bayesian Information Criterion. Adjusted independent variable
148 values were calculated based on regression parameters of the final model to determine
149 the r or R^2 corrected for experiment effect (St-Pierre, 2001).

150

151 3. Results and Discussion

152 Dry matter intake is a major determinant of methane production from cattle (*e.g.*,
153 Bannink et al., 2010). A higher DM intake will generally result in increased amounts
154 of organic matter fermented in the rumen with associated production of VFA and
155 gases. Indeed in the present analysis, DM intake was positively related ($P < 0.001$; $r =$
156 0.84) to methane production with a slope of 23.1 ± 2.38 g methane/kg DM intake. To
157 evaluate dietary mitigation options, variation in the amount of methane produced per
158 unit feed is of more interest than total output of methane because it avoids
159 confounding effects of DM intake on methane production, and because DM intake is
160 known or can be estimated with reasonable accuracy in stall-fed cows. Therefore,
161 methane produced per kg of feed DM was related to individual FA concentrations in
162 milk fat, and results are in Table 2.

163 Consistent with Odongo et al. (2007) and Chilliard et al. (2009), methane
164 production was positively correlated ($P < 0.05$) with C8:0, C10:0, C11:0 and C16:0 (all

165 g/100 g total FA). However, Johnson et al. (2002) did report reduced concentrations
166 of C10:0, C12:0, C14:0 and C16:0 in milk fat upon supplementation with cottonseed
167 and canola seed, and methane production was not affected. These FA are mainly
168 derived from *de novo* synthesis in the mammary gland from acetate and 3-hydroxy
169 butyrate (Bernard et al., 2008). Formation of acetate in the rumen, largely as the result
170 of fermentation of fibre (Bannink et al., 2008), results in the production of hydrogen
171 gas that is used to produce methane by methanogenic archaea. A range of dietary
172 unsaturated FA may reduce methane production (Beauchemin et al., 2009). Since
173 various unsaturated FA are also known to inhibit *de novo* synthesis of FA with 16
174 carbons or less, with the possible exception of C4:0 (Bernard et al., 2008), this may
175 also explain the relationship between methane and *de novo* synthesised FA. Indeed, of
176 FA with 16 carbons or less, only C4:0 tended ($P=0.07$) to be negatively related to
177 methane production.

178 Consistent with theoretical expectations (Vlaeminck and Fievez, 2005), and with
179 experimental data (Chilliard et al., 2009), C14:0 *iso* and C15:0 *iso* in milk fat were
180 positively related ($P=0.02$ and 0.003 , respectively) to methane, but C17:0 *iso* was
181 negatively related ($P=0.02$). Fibrolytic bacteria are enriched in C14:0 *iso* and C15:0
182 *iso*, and an increase in dietary forage to concentrate ratio, which will generally
183 increase methane production, is also associated with higher levels of C14:0 *iso* and
184 C15:0 *iso* in milk fat (Vlaeminck et al., 2006). Odongo et al. (2007) reported a
185 numerical decrease of C17:0 *anteiso* accompanied by a decrease of methane in the
186 myristic supplemented diet. In our study, a positive relationship ($P<0.001$) between
187 methane and C17:0 *anteiso* also occurred. Cabrita et al. (2003) reported a negative
188 relationship between dietary crude protein content and C17:0 *anteiso* content in milk
189 fat, and a positive relationship between dietary fibre content and C17:0 *anteiso*.
190 Because, stoichiometrically, fermentation of protein is associated with a lower
191 methane production compared with fermentation of fibre or sugars (Bannink et al.,
192 2008), such associations between dietary crude protein, fibre and milk C17:0 *anteiso*
193 may explain the positive relationship of this FA with methane.

194 A high propionic acid level in the rumen is associated with low methane
195 production, and propionic acid is a substrate for *de novo* synthesis of C15:0 and
196 C17:0. Thus Vlaeminck and Fievez (2005) expected a negative relationship between
197 these odd chain FA and methane, but Chilliard et al. (2009) reported a positive
198 correlation between these odd chain FA and methane. Odongo et al. (2007) did not

199 find changes in C15:0 and C17:0 contents with changes in methane production. In our
 200 analysis, C15:0 was not related with methane and C17:0 tended ($P=0.07$) to be
 201 positively related. However, *cis-9* C17:1 was negatively related ($P<0.001$) to
 202 methane. *Cis-9* C17:1 is a desaturation product of C17:0 in the mammary gland. The
 203 sum of C17:0 and *cis-9* C17:1 was negatively related ($P=0.03$) to methane production
 204 (results not shown). Supplementation with linseed changed mammary desaturation
 205 activity, which may have caused relationships between milk FA and methane in
 206 Chilliard et al. (2009) to differ from others, and in our findings.

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

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

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

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

227 where individual FA are in g/100 g FA and $R^2 = 0.73$ after correction for the
 228 experiment effect (St-Pierre, 2001) with all parameters $P<0.02$ (see Figure 1 for
 229 observed and predicted relationship and residual methane production). The R^2 of this
 230 equation is lower than the best equation derived by Chilliard et al. (2009). However,
 231 Chilliard et al. (2009) obtained relationships using absolute methane production
 232 (g/day) rather than methane produced/kg feed DM, and they only used diets that

233 varied in supply and availability of linolenic acid, which may have increased the R^2
234 compared with our approach.

235 However our study shows high potential for milk FA to be used as an indicator of
236 methane produced/kg feed consumed. The number of data ($n = 50$) and studies ($n = 3$)
237 used in our analysis were limited and, within experiment there was no variation in
238 type, composition or proportion of dietary forage and concentrate, which may limit
239 application of our equation to other diets. For example, the high contents of *trans*-
240 10+11 C18:1 (10 g/100 g milk total FA) by feeding docosahexaenoic acid enriched
241 diets (Boeckaert et al., 2008) would likely result in predicted methane production
242 being close to zero. More data are needed to confirm relationships between milk FA
243 profile and methane production for a wide range of dietary conditions.

244

245 **4. Conclusions**

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

252

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257

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324 digesta and bacteria. *J. Lipid Res.* 48, 2247–2254.
- 325

326 Table 1
 327 Summary statistics of experimental data used for modelling ($n = 50$) [data from Van
 328 Zijderveld et al. (2011a, 2011b)].
 329

	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 <i>iso</i>	0.153	0.0334	0.093	0.220
<i>cis</i> -9 C14:1	0.963	0.1967	0.566	1.55
C15:0	0.970	0.1482	0.715	1.270
C15:0 <i>iso</i>	0.245	0.0509	0.159	0.458
C15:0 <i>anteiso</i>	0.443	0.0615	0.328	0.573
C16:0	31.30	4.338	21.41	38.46
<i>cis</i> -9 C16:1	1.85	0.299	1.26	2.56
C17:0	0.584	0.1094	0.383	0.774
C17:0 <i>iso</i>	0.203	0.0755	0.113	0.374
C17:0 <i>anteiso</i>	0.227	0.0453	0.102	0.303
<i>cis</i> -9 C17:1	0.228	0.0534	0.121	0.385
C18:0	10.16	1.377	8.11	14.84
<i>trans</i> -6+7+8+9 C18:1	0.359	0.0722	0.249	0.543
<i>trans</i> -10+11 C18:1	1.10	0.411	0.506	2.32
<i>trans</i> -12 C18:1	0.305	0.1660	0.146	0.856
<i>trans</i> -13+14 C18:1	1.13	0.554	0.368	2.45
<i>cis</i> -9 C18:1	18.44	2.158	14.78	24.21
<i>cis</i> -11 C18:1	0.477	0.1029	0.304	0.756
<i>cis</i> -12 C18:1	0.237	0.1124	0.136	0.653
<i>cis</i> -13 C18:1	0.285	0.1181	0.110	0.651
<i>cis</i> -14+ <i>trans</i> -16 C18:1	0.244	0.2104	0.104	0.903
<i>cis</i> -9,12 C18:2	1.30	0.244	0.569	1.82
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.354	0.0938	0.175	0.627
<i>trans</i> -11, <i>cis</i> -15 C18:2	0.228	0.1798	0.100	0.771
<i>cis</i> -9,12,15 C18:3	0.547	0.1566	0.365	1.023
C20:0	0.129	0.0190	0.101	0.173

331 Table 2

332 Linear regression between methane production (g/kg feed DM) and milk fatty acid

333 concentration (g/100 g total fatty acids) with experiment included as random effect.

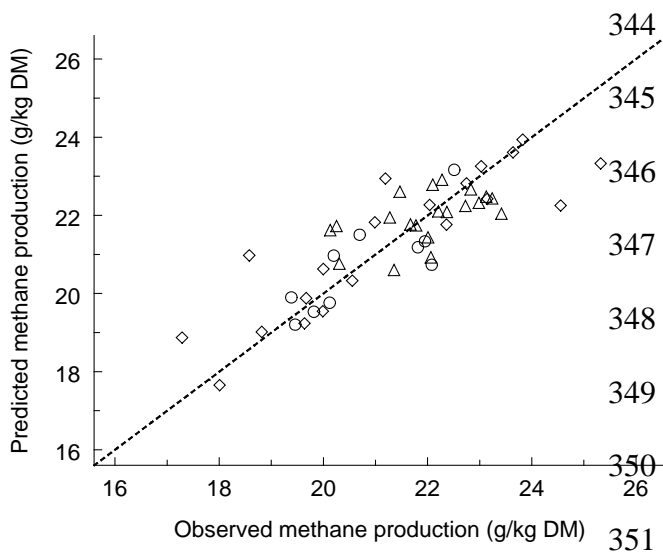
	Intercept	SE	Slope	SE	Slope <i>P</i>	r
C4:0	25.8	2.40	-1.40	0.759	0.07	-0.27
C6:0	18.7	2.18	1.31	1.039	0.21	0.19
C8:0	17.5	1.71	3.17	1.361	0.02	0.32
C10:0	18.6	1.33	1.02	0.463	0.03	0.30
C11:0	17.6	1.21	12.5	3.88	0.002	0.42
C12:0	19.4	1.42	0.641	0.4255	0.14	0.21
C13:0	22.3	1.78	-5.92	13.902	0.67	-0.10
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

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336 Figure 1. Observed and predicted methane production, and residuals (*i.e.*, observed –
 337 predicted) methane production, from the multivariate analysis including experiment as a
 338 discrete class variable. Predicted methane (g/kg DM) = $24.6 + 8.74 \times C17:0 \text{ anteiso} - 1.97 \times$
 339 $trans\text{-}10+11 \text{ C}18:1 - 9.09 \times cis\text{-}11 \text{ C}18:1 + 5.07 \times cis\text{-}13 \text{ C}18:1$ (individual FA in g/100 g of
 340 total FA; $R^2 = 0.73$ after correction for experiment effect (St-Pierre, 2001) with experiment
 341 effect not shown). Δ , experiment 1; \circ , experiment 2; \diamond , experiment 3. The line of unit slope
 342 (dotted line) represents the line of equivalence.

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