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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 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.

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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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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.

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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

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):

224   methane (g/kg DM) = 24.6 ± 1.28 + 8.74 ± 3.581 × C17:0 *anteiso* – 1.97 ± 0.432

225   × *trans*-10+11 C18:1 – 9.09 ± 1.444 × *cis*-11 C18:1 + 5.07 ±

226   1.937 × *cis*-13 C18:1

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.

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257

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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 *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

330

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.
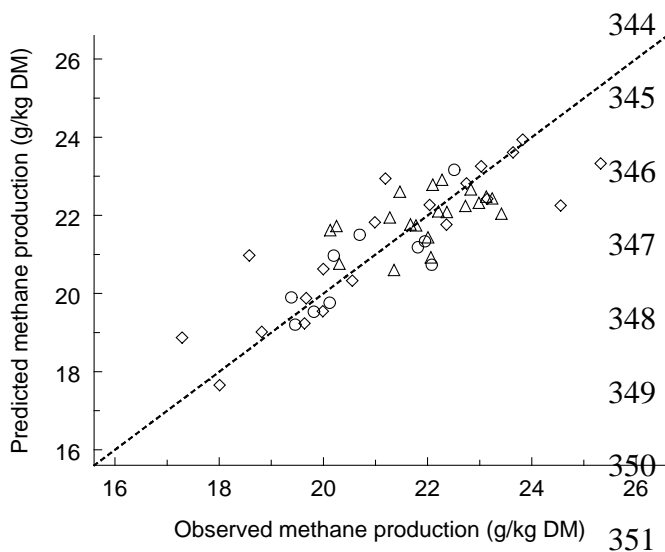
	Intercept	SE	Slope	SE	Slope *P*	r
C4:0	25.8	2.40	-1.40	0.759	0.07	-0.27
C6:0	18.7	2.18	1.31	1.039	0.21	0.19
C8:0	17.5	1.71	3.17	1.361	0.02	0.32
C10:0	18.6	1.33	1.02	0.463	0.03	0.30
C11:0	17.6	1.21	12.5	3.88	0.002	0.42
C12:0	19.4	1.42	0.641	0.4255	0.14	0.21
C13:0	22.3	1.78	-5.92	13.902	0.67	-0.10
C14:0	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

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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 × C17:0 *anteiso* – 1.97 ×

339   *trans*-10+11 C18:1 – 9.09 × *cis*-11 C18:1 + 5.07 × *cis*-13 C18:1 (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; ○, experiment 2; ◊, experiment 3. The line of unit slope

342   (dotted line) represents the line of equivalence.

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