Short communication: Genetic study of methane production predicted from milk fat composition in dairy cows

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ABSTRACT

Dairy cows produce enteric methane, a greenhouse gas with 25 times the global warming potential of CO₂. Breeding could make a permanent, cumulative, and long-term contribution to methane reduction. Due to a lack of accurate, repeatable, individual methane measurements needed for breeding, indicators of methane production based on milk fatty acids have been proposed. The aim of the present study was to quantify the genetic variation for predicted methane yields. The milk fat composition of 1,905 first-lactation Dutch Holstein-Friesian cows was used to investigate 3 different predicted methane yields (g/kg of DMI): Methane₁, Methane₂, and Methane₃. Methane₁ was based on the milk fat proportions of C17:0 anteiso, C18:1 *trans*-10+11, C18:1 *cis*-11, and C18:1 *cis*-13 ($R^2 = 0.73$). Methane₂ was based on C4:0, C18:0, C18:1 *trans*-10+11, and C18:1 *cis*-11 ($R^2 = 0.70$). Methane₃ was based on C4:0, C6:0, and C18:1 *trans*-10+11 ($R^2 = 0.63$). Predicted methane yields were demonstrated to be heritable traits, with heritabilities between 0.12 and 0.44. Breeding can, thus, be used to decrease methane production predicted based on milk fatty acids.

Key words: predicted methane yield, milk fatty acid, dairy cow

Short Communication

Dairy cows produce enteric methane, a greenhouse gas with 25 times the global warming potential of CO₂ (Forster et al., 2007). To reduce methane production, a combination of strategies, such as feeding strategies, should be considered (Patra, 2012; Hristov et al., 2013). Breeding could be a useful addition to any combination of strategies, making a permanent, cumulative, and long-term contribution to reducing methane production.

Breeding for reduced methane production can only be achieved when the trait in question shows variation and part of that variation is heritable. To quantify (genetic) variation, many animals need to be evaluated for the new trait of methane production (Buddle et al., 2011). This evaluation is difficult due to a lack of accurate, repeatable, individual methane measurements. Alternatively, evaluation of methane production can be based on associated traits that are easier to record. Indicators of methane production based on milk FA that are related to rumen conditions and methane production have been proposed by several authors (Chilliard et al., 2009; Dijkstra et al., 2011; Mohammed et al., 2011).

The aim of this study was to apply such indicators to a large number of dairy cows with known milk fat composition to quantify the genetic variation for predicted methane production.

Milk fat composition of morning milk samples from 1,905 first-lactation (63 to 282 d in lactation) Dutch Holstein-Friesian cows from 398 herds was obtained from the Dutch Milk Genomics Initiative. Milk fat composition was determined by gas chromatography as described by Stoop et al. (2008) and expressed in grams per 100 g of fat. Stoop et al. (2008) and Bouwman et al. (2011) previously provided detailed descriptions of the experimental setup.

Three methane prediction equations were derived from the data set of Dijkstra et al. (2011) and applied to our data. The FA in these equations are expressed in grams per 100 g of fat. The first prediction equation (**Methane₁**) was reported by Dijkstra et al. (2011):

$$\text{CH}_4 \text{ (in g/kg of DMI)} = 24.60 + 8.74 \times \text{C17:0 anteiso} \\ - 1.97 \times \text{C18:1 trans-10+11} - 9.09 \times \text{C18:1 cis-11} \\ + 5.07 \times \text{C18:1 cis-13 (Methane}_1\text{)}.$$

The coefficient of determination (R^2) of Methane₁ was 0.73. The R^2 was corrected for experiment effects as described in St-Pierre (2001). A second prediction equation (**Methane₂**) was derived excluding FA that were present in relatively different concentrations (difference

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Table 1. Milk production traits, predicted methane yields based on milk fat composition, and individual milk FA for first-lactation Dutch dairy cows¹

Trait	n	Mean ²	CV ² (%)	σ_p ³	h^2	h^2_{inter}	Herd
Milk production trait							
Milk yield (kg)	1,901	13.41	20	2.64	0.39 (0.10)	0.29 (0.03)	0.29 (0.03)
Fat yield (kg)	1,901	0.58	19	0.11	0.32 (0.09)	0.25 (0.02)	0.25 (0.02)
Fat percentage	1,902	4.36	16	0.70	0.47 (0.10)	0.44 (0.09)	0.07 (0.02)
Protein yield (kg)	1,901	0.47	20	0.09	0.21 (0.08)	0.13 (0.05)	0.38 (0.03)
Protein percentage	1,902	3.51	8	0.29	0.61 (0.12)	0.48 (0.10)	0.20 (0.02)
FPCM ⁴	1,902	14.01	18	2.50	0.33 (0.10)	0.22 (0.07)	0.32 (0.03)
Predicted methane yield (g/kg of DMI)							
Methane1 ⁵	1,838	23.62	6	1.38	0.12 (0.06)	0.05 (0.03)	0.55 (0.02)
Methane2 ⁶	1,898	21.34	6	1.23	0.20 (0.07)	0.12 (0.05)	0.38 (0.03)
Methane3 ⁷	1,898	20.87	4	0.82	0.44 (0.10)	0.30 (0.07)	0.31 (0.03)
Milk FA (g/100 g of fat)							
C4:0	1,898	3.50	8	0.28	0.42 (0.09)	0.35 (0.08)	0.16 (0.02)
C6:0	1,898	2.23	7	0.16	0.46 (0.10)	0.39 (0.09)	0.15 (0.02)
C17:0 anteiso	1,838	0.50	22	0.11	0.11 (0.06)	0.06 (0.03)	0.44 (0.03)
C18:0	1,898	8.73	16	1.40	0.24 (0.07)	0.19 (0.06)	0.19 (0.02)
C18:1 <i>trans</i> -10	1,849	0.22	34	0.07	0.19 (0.08)	0.08 (0.03)	0.56 (0.02)
C18:1 <i>trans</i> -11	1,888	0.77	27	0.21	0.28 (0.09)	0.11 (0.04)	0.60 (0.02)
C18:1 <i>trans</i> -10+11	1,882	1.00	23	0.24	0.30 (0.09)	0.13 (0.04)	0.58 (0.02)
C18:1 <i>cis</i> -11	1,898	0.41	26	0.10	0.20 (0.08)	0.11 (0.05)	0.44 (0.03)
C18:1 <i>cis</i> -13	1,883	0.08	20	0.02	0.29 (0.08)	0.17 (0.05)	0.41 (0.03)

¹Table contains number of observations (n), mean, coefficient of variation, phenotypic standard deviation (σ_p), heritability (h^2 ; $h^2 = \sigma^2_A / (\sigma^2_A + \sigma^2_E)$), interherd heritability (h^2_{inter} ; $h^2_{\text{inter}} = \sigma^2_A / (\sigma^2_{\text{Herd}} + \sigma^2_A + \sigma^2_E)$), and variance explained by the herd (Herd; $\text{Herd} = \sigma^2_{\text{Herd}} / (\sigma^2_{\text{Herd}} + \sigma^2_A + \sigma^2_E)$) with their respective standard errors in parentheses.

²Based on morning milk samples and uncorrected.

³After adjustment by model 1.

⁴Fat- and protein-corrected milk (FPCM) = milk (kg) $\times$ [0.337 + (0.116 $\times$ fat percentage) + (0.06 $\times$ protein percentage)].

⁵Methane1 = 24.60 + 8.74 $\times$ C17:0 anteiso – 1.97 $\times$ C18:1 *trans*-10+11 – 9.09 $\times$ C18:1 *cis*-11 + 5.07 $\times$ C18:1 *cis*-13.

⁶Methane2 = 28.60 – 1.13 $\times$ C4:0 + 0.36 $\times$ C18:0 – 2.57 $\times$ C18:1 *trans*-10+11 – 9.29 $\times$ C18:1 *cis*-11.

⁷Methane3 = 27.13 – 3.04 $\times$ C4:0 + 2.71 $\times$ C6:0 – 1.63 $\times$ C18:1 *trans*-10+11.

between data sets larger than 40%) in our data set compared with that of Dijkstra et al. (2011; $R^2 = 0.70$).

$$\begin{aligned} \text{CH}_4 \text{ (in g/kg of DMI)} &= 28.60 - 1.13 \times \text{C4:0} + 0.36 \\ &\times \text{C18:0} - 2.57 \times \text{C18:1 } \textit{trans}\text{-10+11} - 9.29 \\ &\times \text{C18:1 } \textit{cis}\text{-11 (Methane2)}. \end{aligned}$$

A third prediction equation (**Methane3**) was derived excluding FA with relatively different concentrations and FA with concentrations <1 g/100 g fat in our data set as well as that of Dijkstra et al. (2011; $R^2 = 0.63$):

$$\begin{aligned} \text{CH}_4 \text{ (g/kg of DMI)} &= 27.13 - 3.04 \times \text{C4:0} + 2.71 \\ &\times \text{C6:0} - 1.63 \times \text{C18:1 } \textit{trans}\text{-10+11 (Methane3)}. \end{aligned}$$

Note that C18:1 *trans*-10 and C18:1 *trans*-11 were individually available in our data set and added together before applying the methane prediction equation. Analyses were performed for the predicted methane yields and for individual FA used in the methane prediction equations. Data of 3 cows were removed due to high levels of C18:1 *trans*-10+11 that deviated more than 9 phenotypic standard deviations from the mean

and were considered outliers. Normality of the traits was examined and observations with a residual effect >3.5 based on model 1 were considered outliers and removed. Not all cows had complete data on milk fat composition, resulting in different numbers of observations for the different traits (Table 1).

Genetic parameters and variance components were estimated using the following model in ASReml Version 2.0 (Gilmour et al., 2006):

$$\begin{aligned} Y_{ijkl} &= \mu + b_1 \times \text{dim}_{ijkl} + b_2 \times e^{-0.05 \times \text{dim}_{ijkl}} + b_3 \\ &\times \text{afc}_{ijkl} + b_4 \times \text{afc}_{ijkl}^2 + \text{season}_i + \text{sirecode}_j \\ &+ \text{animal}_k + \text{herd}_l + e_{ijkl} \text{ (model 1)}, \end{aligned}$$

where Y_{ijkl} is the dependent variable; μ is the general mean; dim_{ijkl} is the covariate describing the effect of the number of days in milking; afc_{ijkl} is the covariate describing the effect of age at first calving; season_i is the fixed effect of the class calving season (June–August 2004, September–November 2004, or December 2004–February 2005); sirecode_j is the fixed effect of the difference in genetic level between cows sired by different groups of proven bulls and young bulls, animal_k is

the random additive genetic effect of animal; herd_i is the random effect of herd; and e_{ijkl} is the random error effect. The heritability (h^2) was calculated as follows:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_E^2}.$$

This heritability is often referred to as the intraherd heritability (Heringstad et al., 2006), and these estimates are very similar to heritability estimates from a model with herd as a fixed effect. The interherd heritability (h_{inter}^2) was calculated as follows:

$$h_{\text{inter}}^2 = \frac{\sigma_A^2}{\sigma_{\text{herd}}^2 + \sigma_A^2 + \sigma_E^2}.$$

The proportion of variance explained by herd was calculated as follows:

$$\text{Herd} = \frac{\sigma_{\text{herd}}^2}{\sigma_{\text{herd}}^2 + \sigma_A^2 + \sigma_E^2}.$$

Results are presented in Table 1. The means of methane yields predicted based on milk FA ranged from 20.9 g/kg of DMI for Methane3 to 23.6 g/kg of DMI for Methane1, and the means of the individual FA ranged from 0.08% of total fat for C18:1 *cis*-13 to 8.73% for C18:0. The ranges of predicted methane yields in the current study are similar to the ranges of methane yields in the study of Dijkstra et al. (2011). The ranges of predicted methane yields in this study were 17.3 to 28.2 (g/kg of DMI) for Methane1, 14.6 to 24.4 for Methane2, and 17.7 to 23.5 for Methane3. The range of measured methane (g/kg of DMI) in the study of Dijkstra et al. (2011) was 17.3 to 25.3, and the range of predicted methane (Methane1; g/kg of DMI) was 17.6 to 23.9. Predicted methane yields of this study are also in line with the meta-analysis of van Lingen et al. (2014), with 15.9 to 27.9 (g/kg of DMI) for measured methane and 15.5 to 25.5 (g/kg of DMI) for methane predicted based on milk fat composition.

Methane yields in this study were predicted based on milk FA because milk FA are suggested to be indicative of rumen conditions and, consequently, of methane production in the rumen. Methane and VFA are mainly produced in the rumen as the result of digesting feed in the rumen (Ellis et al., 2008). Certain FA are released directly from the feed and absorbed in the blood. The VFA and FA from the blood can be used in the mammary gland for the production of milk fat. Microorganisms present in the rumen and rumen conditions affect the amount and composition of VFA that are produced

and absorbed, the amount and composition of absorbed FA, and the amount of methane produced (Vlaeminck et al., 2006; Ellis et al., 2008). Therefore, the composition of milk fat has been suggested to be related to methane production in the rumen. Correlations between milk FA and predicted methane production were established successfully (Chilliard et al., 2009; Dijkstra et al., 2011; McCartney et al., 2013), confirming this hypothesis.

Methane prediction equations may not be universal and might be valid only when applied to data sets obtained at similar conditions as the data set from which the prediction equations are derived. The prediction equations for this study were derived from the data set of Dijkstra et al. (2011). The use of these prediction equations on our data set was considered appropriate as conditions and breeds were similar in both data sets. In particular, both studies were based on the Dutch Holstein-Friesian breed and cows in both studies were fed common Dutch diets of mixed grass silage and corn silage. No actual methane data were available to verify that this similarity in conditions justifies the use of methane prediction equations on other data sets.

Methane prediction equations have generally been developed on data sets containing relatively small numbers of observations and limited ranges of dietary treatments. It is further expected that the validation R^2 of the methane prediction equations will be lower than the R^2 reported based on the data set from which the prediction equation was derived. The decreased R^2 is expected to reduce the ability to identify low-methane-producing dairy cows and might affect the genetic parameters of the predicted methane production. The effect of a R^2 lower than 1 on the genetic parameters depends on the values of the genetic parameters in the part that is not explained by the methane prediction equation. When the values of the genetic parameters in the unexplained part are similar to the values of the genetic parameters in the explained part, a small effect is anticipated. The difference between genetic parameters in the explained part and the unexplained part can only be evaluated in data sets containing both measured and predicted methane production for large number of cows.

The coefficient of variation of predicted methane yields ranged from 4% for Methane3 to 6% for Methane1 and Methane2, which is slightly lower than the coefficient of variation of 8% reported by Dijkstra et al. (2011). Milk production traits and individual FA had a higher coefficient of variation, ranging from 7% for C6:0 to 34% for C18:1 *trans*-10. Studies measuring methane production in cows have reported different coefficients of variation depending on the measurement method. For example, Chagunda et al. (2013) reported

a coefficient of variation that ranged between 10% for the methane production of 2 cows in climate respiration chambers to 46% for methane measured with a laser on 2 cows. A low coefficient of variation could cause difficulties in quantifying genetic variance.

The heritabilities of the predicted methane yields ranged from 0.12 for Methane1 to 0.44 for Methane3, and the proportion of variance explained by herd ranged from 0.31 for Methane3 to 0.55 for Methane1. The heritabilities of the individual FA ranged from 0.11 for C17:0 anteiso to 0.46 for C6:0, and the proportion of variance explained by herd ranged from 0.15 for C6:0 to 0.60 for C18:1 *trans*-11. Lassen and Løvendahl (2013) reported a h^2 of 0.21 for the ratio between methane and carbon dioxide measured in an automatic milking system on 683 dairy cows. Pinares-Patiño et al. (2013) measured methane yield (g/kg of DMI) of 1,225 sheep in climate respiration chambers and reported a h^2 of 0.13. de Haas et al. (2011) predicted methane production (g/d) based on feed intake and diet composition for 548 cows, reporting a h^2 of 0.35, whereas predicted methane production corrected for fat and protein corrected milk had a h^2 of 0.58. Actual and predicted methane production shows heritable variation in the literature and in our study; therefore, methane production can be targeted by breeding strategies.

In conclusion, the results show that methane yields predicted based on milk FA (g/kg of DMI; R^2 : 0.73–0.63) are heritable traits, with heritabilities ranging between 0.12 and 0.44. Breeding can, thus, be used to decrease methane production predicted based on milk FA.

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REFERENCES

- Bouwman, A. C., H. Bovenhuis, M. H. P. W. Visker, and J. A. M. van Arendonk. 2011. Genome-wide association of milk fatty acids in Dutch dairy cattle. *BMC Genet.* 12:43.
- Buddle, B. M., M. Denis, G. T. Attwood, E. Altermann, P. H. Janssen, R. S. Ronimus, C. S. Pinares-Patiño, S. Muetzel, and D. Neil Wedlock. 2011. Strategies to reduce methane emissions from farmed ruminants grazing on pasture. *Vet. J.* 188:11–17.
- Chagunda, M. G. G., D. Ross, J. Rooke, T. Yan, J. L. Douglas, L. Poret, N. R. McEwan, P. Teeranavattanakul, and D. J. Roberts. 2013. Measurement of enteric methane from ruminants using a hand-held laser methane detector. *Acta Agric. Scan. A* 63:68–75.
- Chilliard, Y., C. Martin, J. Rouel, and M. Doreau. 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.
- de Haas, Y., J. J. Windig, M. P. L. Calus, J. Dijkstra, M. de Haan, A. Bannink, and R. F. Veerkamp. 2011. Genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. *J. Dairy Sci.* 94:6122–6134.
- Dijkstra, J., S. M. van Zijderveld, J. A. Apajalahti, A. Bannink, W. J. J. Gerrits, J. R. Newbold, H. B. Perdok, and H. Berends. 2011. Relationships between methane production and milk fatty acid profiles in dairy cattle. *Anim. Feed Sci. Technol.* 166–167:590–595.
- Ellis, J., J. Dijkstra, E. Kebreab, A. Bannink, N. Odongo, B. McBride, and J. France. 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. *J. Agric. Sci.* 146:213–233.
- Forster, P., V. Ramaswamy, P. Artaxo, T. Bernsten, R. Betts, D. W. Fahey, J. Haywood, J. Lean, D. C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R. Van Dorland. 2007. Changes in Atmospheric Constituents and in Radiative Forcing. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, ed. Cambridge University Press, Cambridge, UK.
- Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2006. *ASReml User Guide Release 2.0*. VSN International Ltd., Hemel Hempstead, UK.
- Heringstad, B., D. Gianola, Y. M. Chang, J. Ødegård, and G. Klemetsdal. 2006. Genetic associations between clinical mastitis and somatic cell score in early first-lactation cows. *J. Dairy Sci.* 89:2236–2244.
- Hristov, A. N., T. Ott, J. Tricarico, A. Rotz, G. Waghorn, A. Adesogan, J. Dijkstra, F. Montes, J. Oh, E. Kebreab, S. J. Oosting, P. J. Gerber, B. Henderson, H. P. S. Makkar, and J. Firkins. 2013. SPECIAL TOPICS—Mitigation of methane and nitrous oxide emissions from animal operations: III. A review of animal management mitigation options. *J. Anim. Sci.* 91:5095–5113.
- Lassen, J., and P. Løvendahl. 2013. Heritability for enteric methane emission from Danish Holstein cows using a non-invasive FTIR method. *Adv. Anim. Biosci.* 4:280.
- McCartney, C. A., I. D. Bull, and R. J. Dewhurst. 2013. Chemical markers for rumen methanogens and methanogenesis. *Animal* 7(Suppl. 2):409–417.
- Mohammed, R., S. M. McGinn, and K. A. Beauchemin. 2011. Prediction of enteric methane output from milk fatty acid concentrations and rumen fermentation parameters in dairy cows fed sunflower, flax, or canola seeds. *J. Dairy Sci.* 94:6057–6068.
- Patra, A. K. 2012. Enteric methane mitigation technologies for ruminant livestock: A synthesis of current research and future directions. *Environ. Monit. Assess.* 184:1929–1952.
- Pinares-Patiño, C. S., S. M. Hickey, E. A. Young, K. G. Dodds, S. MacLean, G. Molano, E. Sandoval, H. Kjestrup, R. Harland, C. Hunt, N. K. Pickering, and J. C. McEwan. 2013. Heritability estimates of methane emissions from sheep. *Animal* 7(Suppl. 2):316–321.
- St-Pierre, N. R. 2001. Invited Review: Integrating quantitative findings from multiple studies using mixed model methodology. *J. Dairy Sci.* 84:741–755.
- Stoop, W. M., J. A. M. Van Arendonk, J. M. L. Heck, H. J. F. Van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians. *J. Dairy Sci.* 91:385–394.
- van Lingen, H. J., L. A. Crompton, W. H. Hendriks, C. K. Reynolds, and J. Dijkstra. 2014. Meta-analysis of relationships between enteric methane yield and milk fatty acid profile in dairy cattle. *J. Dairy Sci.* 97:7115–7132.
- Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. *Anim. Feed Sci. Technol.* 131:389–417.