Short communication: The effect of linseed oil and *DGAT1* K232A polymorphism on the methane emission prediction potential of milk fatty acids

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ABSTRACT

Several in vivo CH₄ measurement techniques have been developed but are not suitable for precise and accurate large-scale measurements; hence, proxies for CH₄ emissions in dairy cattle have been proposed, including the milk fatty acid (MFA) profile. The aim of the present study was to determine whether recently developed MFA-based prediction equations for CH₄ emission are applicable to dairy cows with different diacylglycerol o-acyltransferase 1 (*DGAT1*) K232A polymorphism and fed diets with and without linseed oil. Data from a crossover design experiment were used, encompassing 2 dietary treatments (i.e., a control diet and a linseed oil diet, with a difference in dietary fat content of 22 g/kg of dry matter) and 24 lactating Holstein-Friesian cows (i.e., 12 cows with *DGAT1* KK genotype and 12 cows with *DGAT1* AA genotype). Enteric CH₄ production was measured in climate respiration chambers and the MFA profile was analyzed using gas chromatography. Observed CH₄ emissions were compared with CH₄ emissions predicted by previously developed MFA-based CH₄ prediction equations. The results indicate that different types of diets (i.e., with or without linseed oil), but not the *DGAT1* K232A polymorphism, affect the ability of previously derived prediction equations to predict CH₄ emission. However, the concordance correlation coefficient was smaller than or equal to 0.30 for both dietary treatments separately, both *DGAT1* genotypes separately, and the complete data set. We therefore concluded that previously derived MFA-based CH₄ prediction equations can neither accurately nor precisely predict CH₄ emissions of dairy cows managed

under strategies differing from those under which the original prediction equations were developed.

Key words: enteric methane production, milk fatty acid, linseed oil, *DGAT1* K232 polymorphism

Short Communication

Enteric CH₄ emission is one of the main targets of greenhouse gas mitigation strategies for the dairy cattle sector (Knapp et al., 2014). Accurate and repeatable measurements of CH₄ emission from individual dairy cows are required to evaluate emission factors used in national inventories of greenhouse gas emissions in agriculture, to assess efficacy of mitigation strategies, and to develop protocols for genetic selection for cows with reduced CH₄ emission (Hammond et al., 2016). The in vivo CH₄ measurement techniques available today are not suitable for precise and accurate large-scale measurements (Hammond et al., 2016). Hence, proxies (i.e., indirect traits related to enteric CH₄ production) have been suggested, including the milk fatty acid (MFA) profile. Castro-Montoya et al. (2017) performed a meta-analysis with data from 9 experiments and concluded that MFA did not reliably predict specific amounts of CH₄ emitted by dairy cows, whereas MFA has modest potential to differentiate individual dairy cows with high or low CH₄ emissions. It has also been shown that the relationship between the MFA profile and CH₄ emission can be affected by dietary composition (Dijkstra et al., 2011; Mohammed et al., 2011). The prediction equation developed on diets with a wide range of additives (Dijkstra et al., 2011) overpredicted CH₄ emission of cows fed sunflower, flax, and canola seeds (Mohammed et al., 2011), and the prediction equations developed on a wide range of diets including a wide variety of additives (van Lingen et al., 2014) could not accurately predict CH₄ emission of dairy cows fed grass- or grass silage-based diets (Dijkstra et al., 2016). The relationship between MFA profiles and CH₄ emission is not only affected by dietary composition, but also by lacta-

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tion stage, as demonstrated by Vanrobays et al. (2016). It is, however, unknown whether host genetics can also influence the relationship between the MFA profile and CH₄ emission.

The diacylglycerol o-acyltransferase 1 (*DGAT1*) K232A polymorphism affects not only major milk components, such as protein and fat content, but also their composition, including the MFA profile (e.g., Bovenhuis et al., 2016). Likewise, feeding linseed oil, a dietary strategy to reduce enteric CH₄ production, affects the MFA profile (e.g., Kliem et al., 2017). The aim of the present study was to determine whether recent MFA-based prediction equations for CH₄ emission are applicable to dairy cows with different *DGAT1* genotypes fed diets with and without linseed oil.

Individual cow data from a crossover design experiment with 2 dietary treatments (i.e., a control diet and a linseed oil diet, with a difference in dietary fat content of 22 g/kg of DM) and 24 lactating Holstein-Friesian cows (i.e., 12 cows with *DGAT1* KK genotype and 12 cows with *DGAT1* AA genotype; each group had 6 primiparous and 6 multiparous cows) were used. The experiment has been described by van Gastelen et al. (2017a) and was in accordance with Dutch law and approved by the Animal Care and Use Committee of Wageningen University & Research (Wageningen, the Netherlands). Dry matter intake, milk production, and enteric CH₄ production of cows were measured in climate respiration chambers, described in detail by Heetkamp et al. (2015) and van Gastelen et al. (2015). Daily CH₄ production was expressed in grams per day, CH₄ yield was expressed in grams per kilogram of DMI,

and CH₄ intensity was expressed in grams per kilogram of fat- and protein-corrected milk (**FPCM**). Methane yield was 22.9 ± 1.52 g/kg of DMI for the control diet, 21.5 ± 1.67 g/kg of DMI for the linseed oil diet, 22.3 ± 1.76 g/kg of DMI for the *DGAT1* AA genotype, and 22.1 ± 1.72 g/kg of DMI for the *DGAT1* KK genotype (mean $\pm$ SD). Dry matter intake was 18.0 ± 3.25 kg for the control diet, 17.5 ± 3.20 kg for the linseed oil diet, 17.8 ± 3.24 kg for the *DGAT1* AA genotype, and 17.6 ± 3.23 kg for the *DGAT1* KK genotype. The FPCM yield was 26.8 ± 5.51 kg for the control diet, 27.3 ± 4.62 kg for the linseed oil diet, 27.2 ± 5.03 kg for the *DGAT1* AA genotype, and 26.9 ± 5.11 kg for the *DGAT1* KK genotype. The MFA profile was elucidated using GC, as described by van Gastelen et al. (2015), and expressed in grams per 100 g of total fatty acids.

The MFA-based CH₄ prediction equations reported by Dijkstra et al. (2011; CH₄ yield only), van Lingen et al. (2014; CH₄ yield and CH₄ intensity only), van Gastelen et al. (2017b), and van Gastelen et al. (2018) were used to predict CH₄ production, yield, and intensity of each individual cow (Table 1). First, the predicted CH₄ emissions, based on the previously mentioned equations, were checked for outliers (i.e., average $\pm 3 \times$ SD), but no outliers were detected. Second, the ability of these equations to predict CH₄ emission of dairy cows with different *DGAT1* genotypes, fed diets with and without linseed oil, was evaluated using the root mean squared prediction error (**RMSPE**) and the concordance correlation coefficient (**CCC**), both described in detail by Ellis et al. (2010). The results of these analyses are shown in Table 2 for all data combined, in Table 3

Table 1. The milk fatty acid-based methane prediction equations used in the present study

Unit of expression and reference	Prediction equation
Methane production (g/d) van Gastelen et al. (2017b) van Gastelen et al. (2018)	CH ₄ (g/d) = $211.2 + 50.4 \times \text{C4:0} + 77.7 \times \text{C14:1 } cis\text{-9} - 82.0 \times \text{C18:1 } trans\text{-11}$ CH ₄ (g/d) = $522 - 98.0 \times \text{C18:1 } trans\text{-15} + \text{C18:1 } cis\text{-11} - 116.7 \times \text{C18:2 } cis\text{-9,trans-11} - 80.9 \times \text{C18:3n-3}$
Methane yield (g/kg of DMI) ¹ Dijkstra et al. (2011)	CH ₄ (g/kg of DMI) = $24.6 + 8.74 \times anteiso \text{ C17:0} - 1.97 \times \text{C18:1 } trans\text{-10+11} - 9.09 \times \text{C18:1 } cis\text{-11} + 5.07 \times \text{C18:1 } cis\text{-13}$
van Lingen et al. (2014)	CH ₄ (g/kg of DMI) = $23.39 + 9.74 \times iso \text{ C16:0} - 1.06 \times \text{C18:1 } trans\text{-10+11} - 1.75 \times \text{C18:2 } cis\text{-9,12}$
van Gastelen et al. (2017b)	CH ₄ (g/kg of DMI) = $27.2 - 7.0 \times \text{C18:2 } cis\text{-9,trans-11}$
van Gastelen et al. (2018)	CH ₄ (g/kg of DMI) = $28.4 - 18.79 \times \text{C18:1 } cis\text{-12} - 5.44 \times \text{C18:3n-3}$
Methane intensity (g/kg of FPCM) ² van Lingen et al. (2014)	CH ₄ (g/kg of FPCM) = $21.13 - 1.38 \times \text{C4:0} + 8.53 \times iso \text{ C16:0} - 0.22 \times \text{C18:1 } cis\text{-9} - 0.59 \times \text{C18:1 } trans\text{-10+11}$
van Gastelen et al. (2017b)	CH ₄ (g/kg of FPCM) = $16.5 + 24.6 \times iso \text{ C15:0} - 15.5 \times \text{C17:0} + 52.4 \times \text{C22:0}$
van Gastelen et al. (2018)	CH ₄ (g/kg of FPCM) = $13.7 - 1.33 \times \text{C4:0} + 32.00 \times iso \text{ C15:0} - 4.12 \times \text{C18:3n-3} - 35.44 \times \text{C20:1 } cis\text{-11} + 27.62 \times \text{C22:5n-3}$

¹Dry matter intake in kg/d.

²Fat- and protein-corrected milk (kg/d) = $[0.337 + 0.116 \times \text{fat (g/100 g of milk)} + 0.06 \times \text{protein (g/100 g of milk)}] \times \text{milk yield (kg/d)}$ (CVB, 2012).

Table 2. The root mean squared prediction error (RMSPE) and concordance correlation coefficient (CCC) results of the milk fatty acid-based methane prediction models (complete data set, $n = 48$)

Item	RMSPE (unit) ¹	CCC ²	r^3	C_b^4	v^5	μ^6
Methane production (g/d)						
van Gastelen et al. (2017b)	122	0.20	0.32	0.65	0.75	1.003
van Gastelen et al. (2018)	132	0.17	0.33	0.52	0.82	1.355
Methane yield (g/kg of DMI ⁷)						
Dijkstra et al. (2011)	7.8	0.11	0.47	0.24	0.36	2.308
van Lingen et al. (2014)	2.6	0.30	0.55	0.56	1.10	1.260
van Gastelen et al. (2017b)	2.6	0.29	0.32	0.92	0.67	0.011
van Gastelen et al. (2018)	6.0	0.15	0.41	0.37	0.39	1.576
Methane intensity (g/kg of FPCM ⁸)						
van Lingen et al. (2014)	3.1	0.23	0.46	0.51	1.14	1.378
van Gastelen et al. (2017b)	2.4	0.12	0.18	0.65	1.65	-0.892
van Gastelen et al. (2018)	3.5	0.29	0.38	0.78	0.53	0.389

¹Root mean squared prediction error expressed in g/d, g/kg of DMI, and g/kg of fat- and protein-corrected milk (FPCM) for methane production, yield, and intensity, respectively.

²Concordance correlation coefficient, where $CCC = r \times C_b$.

³Pearson correlation coefficient; a measure of precision.

⁴Bias correction factor; a measure of accuracy.

⁵Scale shift; change in SD between predicted and observed methane emission.

⁶Location shift; if positive, underprediction; if negative, overprediction.

⁷Dry matter intake in kg/d.

⁸Fat- and protein-corrected milk (kg/d) = $[0.337 + 0.116 \times \text{fat (g/100 g of milk)} + 0.06 \times \text{protein (g/100 g of milk)}] \times \text{milk yield (kg/d)}$ (CVB, 2012).

for the control and linseed oil diet separately, and in Table 4 for the *DGAT1* KK genotype and *DGAT1* AA genotype separately.

With respect to the complete data set ($n = 48$), the prediction equations did not predict CH₄ emissions satisfactorily. The RMSPE of CH₄ production was 122 to 132 g/d, the RMSPE of CH₄ yield was 2.6 to 7.8 g/kg of DMI, and the RMSPE of CH₄ intensity was 2.4 to 3.5 g/kg of FPCM. The CCC ranged from 0.17 to 0.20 for CH₄ production, from 0.11 to 0.30 for CH₄ yield, and from 0.12 to 0.29 for CH₄ intensity. These low CCC values were composed of consistently low values for precision ($r < 0.55$), whereas values for accuracy (C_b) ranged from low (0.24) to high (0.92). Most of the CH₄ prediction equations had a positive μ value (i.e., location shift), indicating a general underprediction of CH₄ emission, with the exception of the prediction equations of van Gastelen et al. (2017b) for CH₄ yield (small bias only; consistent with high C_b value) and CH₄ intensity (overprediction of CH₄ emission). As discussed by Ellis et al. (2010), the variable v (i.e., scale shift) measures the relative difference in standard deviation between predicted and observed values. The prediction equations of van Lingen et al. (2014) for CH₄ yield and CH₄ intensity had a scale shift close to 1.0, whereas the scale shift of the prediction equation of van Gastelen et al. (2017b) for CH₄ intensity was 1.65. The latter suggests that the variation in the predicted CH₄ emissions was smaller than the variation in the observed CH₄ emissions. Most of the CH₄ prediction equations, however,

had a scale shift smaller than 1.0, suggesting that the variation in the predicted CH₄ emissions was larger than the variation in the observed CH₄ emissions.

Upon dividing the data set into separate data sets representing the control diet ($n = 24$) and the linseed oil-supplemented diet ($n = 24$), the equations predicting CH₄ emissions performed even less satisfactorily. This is evident by the lower, and sometimes negative, CCC values for both control and linseed oil data sets relative to the complete data set. For the control diet, the scale shift (v) increased considerably (i.e., ranging from 1.18 to 3.47) relative to the complete data set, indicating the inability of the CH₄ prediction equations to predict the range of observed CH₄ emissions. For the linseed oil-supplemented diet, the scale shift varied between 0.57 and 1.87, which is of similar magnitude as for the complete data set. The larger location shift (μ) values for the linseed oil supplemented diet relative to the complete data set, indicated a biased (in general underpredicted) CH₄ prediction. Overall, these results suggest that the dietary composition affects the capability of previously developed MFA-based CH₄ prediction equations to predict CH₄ emissions of dairy cows.

It should be noted that one would most likely interpret the RMSPE results differently than the CCC results for the control diet. Both RMSPE and CCC decrease for the control diet relative to the complete data set (Table 2 and 3). This is contradictory, because a decrease in RMSPE implies an improvement of the CH₄ prediction (Bibby and Toutenburg, 1977), whereas

Table 3. The root mean squared prediction error (RMSPE) and concordance correlation coefficient (CCC) results of the milk fatty acid-based methane prediction models (control diet and linseed oil diet, both n = 24)

Item	RMSPE (unit) ¹	CCC ²	<i>r</i> ³	<i>C_b</i> ⁴	<i>v</i> ⁵	<i>μ</i> ⁶
Control diet						
Methane production (g/d)						
van Gastelen et al. (2017b)	65	0.20	0.40	0.50	3.47	0.506
van Gastelen et al. (2018)	74	0.26	0.54	0.49	2.53	1.072
Methane yield (g/kg of DMI ⁷)						
Dijkstra et al. (2011)	3.4	0.05	0.17	0.33	1.18	2.018
van Lingen et al. (2014)	2.1	0.16	0.35	0.46	1.98	1.363
van Gastelen et al. (2017b)	2.0	0.11	0.24	0.45	2.24	−1.331
van Gastelen et al. (2018)	1.8	0.16	0.23	0.70	1.64	0.773
Methane intensity (g/kg of FPCM ⁸)						
van Lingen et al. (2014)	2.8	0.07	0.17	0.42	2.49	1.381
van Gastelen et al. (2017b)	2.4	0.04	0.05	0.74	1.99	−0.453
van Gastelen et al. (2018)	2.8	0.04	0.05	0.82	1.28	−0.615
Linseed oil diet						
Methane production (g/d)						
van Gastelen et al. (2017b)	159	0.05	0.17	0.29	0.98	2.189
van Gastelen et al. (2018)	172	0.01	0.07	0.20	1.29	2.808
Methane yield (g/kg of DMI)						
Dijkstra et al. (2011)	10.5	0.02	0.43	0.06	0.84	5.696
van Lingen et al. (2014)	3.0	0.16	0.49	0.33	1.53	1.954
van Gastelen et al. (2017b)	3.0	−0.04	−0.05	0.76	0.80	0.760
van Gastelen et al. (2018)	8.2	0.01	0.14	0.11	0.77	4.100
Methane intensity (g/kg of FPCM)						
van Lingen et al. (2014)	3.4	0.02	0.13	0.15	1.86	3.307
van Gastelen et al. (2017b)	2.5	0.11	0.25	0.45	1.03	−1.565
van Gastelen et al. (2018)	4.1	−0.01	−0.04	0.34	0.57	1.873

¹Root mean squared prediction error expressed in g/d, g/kg of DMI, and g/kg of fat- and protein-corrected milk (FPCM) for methane production, yield, and intensity, respectively.

²Concordance correlation coefficient, where $CCC = r \times C_b$.

³Pearson correlation coefficient; a measure of precision.

⁴Bias correction factor; a measure of accuracy.

⁵Scale shift; change in SD between predicted and observed methane emission.

⁶Location shift; if positive, underprediction; if negative, overprediction.

⁷Dry matter intake in kg/d.

⁸Fat- and protein-corrected milk (kg/d) = $[0.337 + 0.116 \times \text{fat (g/100 g of milk)} + 0.06 \times \text{protein (g/100 g of milk)}] \times \text{milk yield (kg/d)}$ (CVB, 2012).

a decrease in CCC implies a poorer CH₄ prediction (Lin, 1989). However, as demonstrated by Ellis et al. (2010) upon evaluating in vivo farm models for CH₄ prediction, when prediction equations are unable to describe adequate amounts of the observed variation, CCC analysis is likely the better evaluation tool. This also applies to the CH₄ prediction equations applied to the control diet in the current study, with relatively high *v* values (ranging from 1.18 to 3.47; Table 3) indicating the inability of the CH₄ prediction equations to predict the range of observed CH₄ emissions.

Dividing the data set into separate data sets representing the *DGAT1* KK genotype (n = 24) and AA genotype (n = 24) hardly affected the RMSPE and CCC results relative to the complete data set. This indicates that the prediction potential of previously developed MFA-based CH₄ equations was not affected by the *DGAT1* K232A polymorphism.

The results suggests that dietary composition (i.e., with or without supplementation of linseed oil) af-

fects the prediction potential of previously derived MFA-based CH₄ prediction equations, whereas the effect of *DGAT1* K232A polymorphism seems small. This may be related to the effect of linseed oil and *DGAT1* K232A polymorphism on the MFA profile. For example, the prediction equation of van Lingen et al. (2014) for CH₄ yield included *iso* C16:0 (positive), C18:1 *trans*-10+11 (negative), and C18:2n-6 (negative) as explanatory variables. van Gastelen et al. (2017a) demonstrated that the first of these 2 MFA were substantially affected by linseed oil supplementation. To the contrary, according to van Gastelen et al. (2017a), the *DGAT1* K232A polymorphism only affected C18:2n-6, whereas the other 2 MFA were not affected. The same patterns was observed for the prediction equation of van Gastelen et al. (2018), which included C18:1 *trans*-15 + C18:1 *cis*-11, C18:2 *cis*-9,*trans*-11, and C18:3n-3 (all negatively related) as explanatory variables for CH₄ production (g/d). van Gastelen et al. (2017a) demonstrated that these 3 MFA substantially

increased upon linseed oil supplementation. Contrary to the effect of dietary composition, only C18:3n-3 was significantly affected by the *DGAT1* K232A polymorphism, whereas the other 2 MFA were unaffected (van Gastelen et al., 2017a). The effect of linseed oil supplementation on these specific MFA might explain why the CH₄ prediction potential of the MFA-based prediction equation of van Lingen et al. (2014) and van Gastelen et al. (2018) was affected by dietary composition. The minor and no effect of *DGAT1* K232A polymorphism on these specific MFA might explain why the MFA-based prediction equation of van Lingen et al. (2014) and van Gastelen et al. (2018) predicted CH₄ emission equally well for the complete data set and the data sets for the *DGAT1* KK and *DGAT1* AA genotype separately.

Overall, the present results indicate that dietary composition (i.e., with or without linseed oil), but not the *DGAT1* K232A polymorphism, affect the ability of previously derived MFA-based CH₄ prediction equations to predict CH₄ emission of dairy cows. This dietary

effect on the ability to predict CH₄ emissions seems to be the result of diet-induced changes in the relationship between MFA profiles and enteric CH₄ production. Hence, we concluded that CH₄ prediction equations may not be universal and might be valid only when applied to dairy cows managed under similar strategies as those under which the original prediction equations were developed.

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Table 4. The root mean squared prediction error (RMSPE) and concordance correlation coefficient (CCC) results of the MFA-based methane prediction models (*DGAT1* AA genotype and *DGAT1* KK genotype, both n = 24)

Item	RMSPE (unit) ¹	CCC ²	r ³	C _b ⁴	v ⁵	μ ⁶
<i>DGAT1</i> KK genotype						
Methane production (g/d)						
van Gastelen et al. (2017b)	114	0.20	0.30	0.68	0.70	0.897
van Gastelen et al. (2018)	118	0.18	0.32	0.56	0.83	1.244
Methane yield (g/kg of DMI ⁷)						
Dijkstra et al. (2011)	7.6	0.10	0.38	0.25	0.37	2.229
van Lingen et al. (2014)	2.4	0.33	0.54	0.62	1.09	1.101
van Gastelen et al. (2017b)	2.4	0.29	0.30	0.94	0.73	-0.167
van Gastelen et al. (2018)	5.2	0.15	0.35	0.43	0.42	1.378
Methane intensity (g/kg of FPCM ⁸)						
van Lingen et al. (2014)	2.9	0.33	0.56	0.58	1.31	1.160
van Gastelen et al. (2017b)	2.7	0.09	0.17	0.54	2.15	-1.047
van Gastelen et al. (2018)	3.5	0.38	0.45	0.84	0.57	0.252
<i>DGAT1</i> AA genotype						
Methane production (g/d)						
van Gastelen et al. (2017b)	129	0.21	0.34	0.62	0.78	1.088
van Gastelen et al. (2018)	145	0.18	0.36	0.49	0.83	1.443
Methane yield (g/kg of DMI)						
Dijkstra et al. (2011)	8.1	0.13	0.55	0.23	0.36	2.343
van Lingen et al. (2014)	2.8	0.29	0.58	0.50	1.13	1.400
van Gastelen et al. (2017b)	2.7	0.31	0.35	0.89	0.63	0.173
van Gastelen et al. (2018)	6.6	0.16	0.49	0.32	0.36	1.749
Methane intensity (g/kg of FPCM)						
van Lingen et al. (2014)	3.3	0.15	0.36	0.43	0.97	1.634
van Gastelen et al. (2017b)	2.1	0.17	0.22	0.76	1.29	-0.758
van Gastelen et al. (2018)	3.6	0.19	0.27	0.70	0.48	0.555

¹Root mean squared prediction error expressed in g/d, g/kg of DMI, and g/kg of fat- and protein-corrected milk (FPCM) for methane production, yield, and intensity, respectively.

²Concordance correlation coefficient, where $CCC = r \times C_b$.

³Pearson correlation coefficient; a measure of precision.

⁴Bias correction factor; a measure of accuracy.

⁵Scale shift; change in SD between predicted and observed methane emission.

⁶Location shift; if positive, underprediction; if negative, overprediction.

⁷Dry matter intake in kg/d.

⁸Fat- and protein-corrected milk (kg/d) = $[0.337 + 0.116 \times \text{fat (g/100 g of milk)} + 0.06 \times \text{protein (g/100 g of milk)}] \times \text{milk yield (kg/d)}$ (CVB, 2012).

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REFERENCES

- Bibby, J., and T. Toutenburg. 1977. Prediction and Improved Estimation in Linear Models. John Wiley & Sons, Chichester, UK.
- Bovenhuis, H., M. H. P. W. Visker, N. A. Poulsen, J. Sehested, H. J. F. van Valenberg, J. A. M. van Arendonk, L. B. Larsen, and A. J. Buitenhuis. 2016. Effect of the diacylglycerol o-acyltransferase 1 (DGAT1) K232A polymorphism on fatty acid, protein, and mineral composition of dairy cattle milk. *J. Dairy Sci.* 99:3113–3123.
- Castro-Montoya, J. M., N. Peiren, J. Veneman, B. De Baets, S. De Campeneere, and V. Fievez. 2017. Predictions of methane emission levels and categories based on milk fatty acid profiles from dairy cows. *Animal* 11:1153–1162.
- CVB. 2012. Chemische samenstellingen en nutritionele waarden van voedermiddelen (in Dutch). CVB (Centraal Veevoederbureau), The Hague, the Netherlands.
- Dijkstra, J., S. van Gastelen, E. C. Antunes-Fernandes, D. Warner, B. Hatew, G. Klop, S. C. Podesta, H. J. van Lingen, K. A. Hettinga, and A. Bannink. 2016. Relationships between milk fatty acid profiles and enteric methane production in dairy cattle fed grass- or grass silage-based diets. *Anim. Prod. Sci.* 56:541–548.
- 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. L., A. Bannink, J. France, E. Kebreab, and J. Dijkstra. 2010. Evaluation of enteric methane prediction equations for dairy cows used in whole farm models. *Glob. Change Biol.* 16:3246–3256.
- Hammond, K. J., L. A. Crompton, A. Bannink, J. Dijkstra, D. R. Yáñez-Ruiz, P. O'Kiely, E. Kebreab, M. A. Eugène, Z. Yu, K. J. Shingfield, A. Schwarm, A. N. Hristov, and C. K. Reynolds. 2016. Review of current in vivo measurements techniques for quantifying enteric methane emission from ruminants. *Anim. Feed Sci. Technol.* 219:13–30.
- Heetkamp, M. J. W., S. J. J. Alferink, T. Zandstra, P. Hendriks, H. van den Brand, and W. J. J. Gerrits. 2015. Design of climate respiration chambers, adjustable to the metabolic mass of subjects. Pages 35–56 in *Indirect Calorimetry. Techniques, Computations and Applications*. W. J. J. Gerrits and E. Labussière, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Kliem, K. E., D. J. Humphries, C. K. Reynolds, and R. Morgan. 2017. Effect of oilseed type on milk fatty acid composition of individual cows, and also bulk tank milk fatty acid composition from commercial farms. *Animal* 11:354–364.
- Knapp, J. R., G. L. Laur, P. A. Vadas, W. P. Weiss, and J. M. Tricarico. 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *J. Dairy Sci.* 97:3231–3261.
- Lin, L. I. K. 1989. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 45:255–268.
- 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.
- van Gastelen, S., E. C. Antunes-Fernandes, K. A. Hettinga, and J. Dijkstra. 2017b. Relationships between methane emission of Holstein Friesian dairy cows and fatty acids, volatile metabolites and non-volatile metabolites in milk. *Animal* 11:1539–1548.
- van Gastelen, S., E. C. Antunes-Fernandes, K. A. Hettinga, and J. Dijkstra. 2018. The relationship between milk metabolome and methane emission of Holstein Friesian dairy cows—Metabolic interpretation and prediction potential. *J. Dairy Sci.* 101:2110–2126. <https://doi.org/10.3168/jds.2017-13334>.
- van Gastelen, S., E. C. Antunes-Fernandes, K. A. Hettinga, G. Klop, S. J. J. Alferink, W. H. Hendriks, and J. Dijkstra. 2015. Enteric methane production, rumen volatile fatty acid concentrations, and milk fatty acid composition in lactating Holstein-Friesian cows fed grass silage- or corn silage-based diets. *J. Dairy Sci.* 98:1915–1927.
- van Gastelen, S., M. H. P. W. Visker, J. E. Edwards, E. C. Antunes-Fernandes, K. A. Hettinga, S. J. J. Alferink, W. H. Hendriks, H. Bovenhuis, H. Smidt, and J. Dijkstra. 2017a. Linseed oil and DGAT1 K232A polymorphism: Effects on methane emission, energy and N metabolism, lactation performance, ruminal fermentation, and rumen microbial composition of Holstein-Friesian cows. *J. Dairy Sci.* 100:8939–8957.
- 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.
- Vanrobays, M.-L., C. Bastin, J. Vandenplas, H. Hammami, H. Soyeurt, A. Vanlierde, F. Dehareng, E. Froidmont, and N. Gengler. 2016. Changes throughout lactation in phenotypic and genetic correlations between methane emissions and milk fatty acid contents predicted from milk mid-infrared spectra. *J. Dairy Sci.* 99:7247–7260.