Exploring different definitions of methane phenotypes in Dutch Holstein Cattle

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Abstract One of the most promising strategies to permanently reduce methane emissions in dairy cattle is through genomic selection, where the primary goal is to identify and selectively breed low-emitting ruminants. An important step is to define which trait definition to use. Several methane phenotypes have been proposed in recent years. When measuring methane using breath analyzers (sniffers), 2 commonly used phenotypes are methane concentration (CH_4c; ppm) and methane production (CH_4p; g/d). However, for both phenotypes different definitions exist. For example, CH_4c, the average per visit is commonly used, but other phenotypes involving the identification of "eructation" peaks have been suggested. For CH_4p several formulas are available to calculate it, using CH_4c or the ratio between CH_4c and CO_2 concentrations as input, together with other production traits. Additionally, methane intensity, another phenotype of interest, is the amount of methane produced per milk unit (kg). Therefore, the aims of this study were to: 1) Estimate genetic parameters for 11 distinct phenotypes, including 3 CH_4c definitions, carbon dioxide concentration per visit (CO_2c), the ratio between CH_4c and CO_2c, 5 phenotypes for CH_4p based on different formulas (Madsen, Chagunda, 2 of Kjeldsen, and IPCC-Tier2 as benchmark), and methane intensity (MeI). 2) Estimate genetic correlations between these methane phenotypes and milk yield (MY) and body weight (BW). A total of 149,726 sniffer (CH_4c and CO_2c) records were available from 7,600 Dutch Holstein cows measured between 2019 and 2024 on 68 farms. Data were analyzed with an animal repeatability model with fixed effects, including herd-year-season, week of lactation and lactation number with age of cow at calving nested. Estimated heritability values ranged between 0.16 and 0.30 for CH_4c phenotypes, with the number of eructation peaks having the highest heritability. For CH_4p, heritabilities ranged between 0.03 to 0.27, with Tier2 having the highest value. Heritabilities for ratio and CO_2c were 0.08 and 0.13, respectively. Genetic correlations between CH_4c phenotypes were moderate to highly positive, ranging between 0.49 and 0.85. Likewise, highly positive genetic correlations (between 0.89 and 1) were estimated for CH_4p phenotypes, except for Tier2, which presented correlations between 0.04 to 0.37 with the other CH_4p phenotypes. Both mean CH_4c (avg) and sum of max peaks for CH_4c (speaks) had moderate to high positive correlations (0.36–0.95) with the majority of the CH_4p phenotypes (except Tier2). Methane intensity had moderate to high positive genetic correlations (0.38 to 0.80) with the majority of the phenotypes except for Kjeldsen2 (0.03) and Tier2 (−0.90). Milk yield had positive genetic correlations with all the methane phenotypes (0.04 to 0.94) except for MeI (−0.66) and Kjeldsen2 (−0.69). Body weight had close to zero genetic correlations with CH_4c phenotypes (−0.09 to 0.07), and moderate positive to moderate negative correlation (−0.72 to 0.57) for CH_4p phenotypes. Given their strong correlations with the other methane phenotypes, close to zero correlation with body weight, and no induced dependencies with BW and MY, as seen with the CH_4p phenotypes, avg and speaks appear to suitable proxies for methane emissions when using sniffers.

Methane emissions in dairy cattle have been investigated intensively over the past decade due to the 2030–2050 climate targets, where the EU aims for a 55% reduction up to neutrality in greenhouse gas emissions (EEA, 2023). One of the mitigation options is through genetic/genomic selection, where the primary goal is to identify and selectively breed low-emitting animals. As a result, several countries are currently monitoring enteric methane from ruminants. A widely employed phenotyping method involves breath sampling during milking by a breath analyzer device commonly referred to as "sniffer." This device samples breath at regular intervals (often between 1 and 15 s) during milking, reporting methane concentration (CH_4c) in parts per million (ppm). While CH_4c has been proposed as an indicator for gross methane emissions (referred to as methane production (CH_4p); CH_4 g/d) due to its high correlation (0.76; van Breukelen et al., 2022), there is a lack of consensus on which phenotype to use for estimating breeding values. Several phenotypes have been proposed over the past years; one largely used is the average CH_4c per visit or per minute averaged daily or weekly, but other CH_4c phenotypes involving the identification of "eructation" peaks have also been suggested (Garnsworthy et al., 2012; Rey et al., 2019; Reintke et al., 2020). Both studies that investigated peaks used CH_4c measured by mobile laser methane detectors (LMDs). Rey et al. (2019) defined the peaks as the number of peaks or eructation events presented in 5 min. Reintke et al. (2020) proposed the sum of CH_4c per minute during eructation peaks, maximum CH_4c during eructation peaks, and the number of eructation events per minute as possible CH_4c phenotypes in ewe breeding. The rationale behind the use of eructation peaks as a phenotype is to try to disentangle CH_4c that comes from a respiration event from the one that comes from an eructation event (i.e., the peaks). Previous studies by nutritionists (Blaxter and Joyce, 1963 and Murray et al., 1976) showed that

The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-25. Nonstandard abbreviations are available in the Notes.

approximately 17% of the methane exhaled originated from the lungs, while the remaining 83% was produced by eructation. Additionally, eructation peak detection could adjust for barn ambient CH_4c, but also for build-up of CH_4c in the AMS feed bin during milking (Bell, et al., 2014). While there is no published literature on the heritability of eructation peaks in cows, several studies have reported the detection of methane peaks using sniffers (Rey et al., 2019; Hardan et al., 2022). Additionally, sniffers traditionally rely on formulas to "convert" CH_4c in ppm to CH_4p in g/d . Currently, there are several formulas available to calculate CH_4p using CH_4c and body weight (BW; Chagunda et al., 2009) or predicting carbon dioxide (CO_2 g/d; via heat production using BW, energy-corrected milk, and days in pregnancy) and multiplying it by the ratio between CH_4c and CO_2c to predict CH_4p (Pedersen et al., 2008; Madsen et al., 2010). Despite some of these formulas being extensively used, they were originally developed to predict CH_4p in barns, not individual animal CH_4p. Recently, Kjeldsen et al. (2024) developed 3 formulas to predict CO_2 g/d using an international data set of more than 2 thousand cows with recorded CH_4p using either respiration chambers or GreenFeed. Finally, the IPCC (2019) has proposed several approaches (Tier1 to 3) to calculate methane emission factors per species, where the Tier2 formula is extensively used for dairy cattle, as it uses an energy balance approach. Therefore, the aims of this study were to: 1) Estimate genetic parameters for 11 distinct phenotypes, including 3 CH_4c definitions, CO_2 concentration (CO_2c) per visit, the ratio between average CH_4c and CO_2c, 5 phenotypes for CH_4p based on different formulas (Madsen, Chagunda, Kjeldsen, and Tier2), and methane intensity (MeI); and 2) Estimate genetic correlations between these methane phenotypes and milk yield (MY, kg) and BW.

The original data included a total of 149,726 sniffer CH_4c and CO_2c records from roughly 7,600 primi- and multiparous Dutch Holstein cows. Data has been previously described in van Breukelen et al. (2023; 2024). Methane concentration was measured using sniffers located in automated milking systems (AMS) (WD-WUR v2.0, Carltech BV), sampling CH_4c and CO_2c every 5 s. Based on the sniffer CH_4c and CO_2c (ppm) measurements, 11 phenotypes were derived. We divided the phenotypes into groups: CH_4c in ppm, CH_4p in g/d and methane intensity (MeI in g CH_4/ kg MY). Methane concentration phenotypes included 1) average CH_4c per visit (**avg**), 2) number of eructation peaks per minute (**npeaks**), 3) sum of maximum eructation peaks defined as the sum of the average of the 2 top values within each peak (for all peaks within visit; **speaks**). Methane production phenotypes were calculated with 5 formulas available in the literature: 4) **Madsen** (Madsen et al., 2010) based on the prediction of CO_2 using ECM and BW to posteriorly multiply it by ratio; 5) **Chagunda** (Chagunda et al., 2009) based on the tidal volume that uses BW as a predictor and the average CH_4c; 6) and 7) **Kjeldsen2** and **Kjeldsen3** (Kjeldsen et al., 2024) based on the prediction of CO_2 using ECM and BW or only ECM, and multiplied by the ratio between CH_4c and CO_2c; 8) **Tier2** (IPCC, 2019) based on the prediction of CH_4p using MY and BW. Tier 2 was used as a benchmark scenario incorporating information on MY, body weight BW, and diet, without relying on sniffer-based measurements, and was not treated as a selection phenotype. Additionally, we included as phenotypes 9) the **ratio** of CH_4c to CO_2c, 10) CO_2 concentration (**CO_2c**), and 11) methane intensity (**MeI**) defined as CH_4p divided by MY (g CH_4p / kg MY), where CH_4p was calculated using the Kjeldsen3 formula (as it was

the phenotype with the largest number of records and animals). Each of the 11 phenotypes were calculated at visit level. Weekly CH_4c and CH_4p phenotypes were calculated averaging visit phenotypes (with a minimum number of 5 visits) per calendar week. All CH_4c phenotypes had a background level subtracted, where the background was calculated as the 5 lowest measurements during the entire visit, with the purpose to account for the CH_4c level in the barn. Furthermore, visit CH_4c and CH_4p was defined as the concentration present in a window of 240 s (between 60 and 300 s after the entrance time of the cow in the AMS). The first 60 s were removed to account for the gas traveling through the tube until the gas sensor. Visits were cut at 300 s and visits with less that 300s were discarded to avoid erratic data due to head movement as shown by Løvendahl, et al. (2024), as pellets are dropped in the AMS and consumed (mainly) in the first minutes of the milking. Eructation peaks were defined using a self-written function in R to detect peaks. This approach detects local maxima by computing the first and second differences of the signal (0.0005). The function calculates the change between consecutive values. A peak is defined as a point where the signal transitions from increasing to decreasing — i.e., where the first derivative is positive followed by a negative slope. Once eructation peaks were defined, 2 phenotypes were calculated, a) number of peaks per minute, that was the total number of peaks per visit divided by visit length and b) the 2 maximum values per peak were detected, averaged and then summed across all the peaks per visit. The majority of animals had MY and milk content information, however, only ~10% of the animals had BW. For this reason, phenotypes that used BW or metabolic BW (MBW) had a smaller number of records and animals. The number of records and animals per phenotype after editing is presented in Table 1.

The CH_4p phenotypes were calculated using the following formulas:

Madsen: CO_2 (g/d) = 180 × 24 × (5.6 MBW + 22 ECM + 1.6 × 10^{-5} × num days in gestation)

Chagunda: CH_4p (g/d) = mean $CH_4c/10^6 \mu L$ × tidal volume(L/breath) × 30 breaths/min × 1,440 min/d × 16.04 (g/mol)/22.4(L/mol)

where TV(L) = 7.5Ml / kgofLW × LW(kg)/1,000(mL/L)

Kjeldsen2

CO_2 (g/d) = −6134 + (213 × ECM) + (126 × MBW) + (52.5 × Milk CF) + (−5.13 × DIM)+ 2117 + (−0.122 × DIM × Diet CF) + (0.386 × ECM × DIM) + (−1.18 × ECM × MBW) + (−0.614 × Milk CF × MBW) + (−5.96 × MBW) + (−1.18 × DIM) + (−0.614 × MBW)

where Milk CF is crude fat in milk, Diet CF is crude fat in the diet.

Kjeldsen3

CO_2 (g/d) = 8781 + (80.3 × ECM) + (−4.66 × DIM) + −49.0 + parity coefficient + breed-parity coefficient + (−0.149 × DIM × Diet CF) + (0.338 × ECM × DIM) + (206 × DIM) + (Milk CF-

parity coefficient × Milk CF)

where parity coefficients (Kjeldsen et al., 2024) are 0 for first, 511 for second and 1587 for third and higher parities, breed-parity coefficients are for Holstein 0 for first, 775 for second and 803 for third and higher, and MilkCF-parity coefficients are −4.18 for first, −10.5 for second and −28.8 for third and higher.

Tier2 (Equation 10.21)

$$CH_4p \ (g/d) = (GE * 0.065 / 55.65) * 1000$$

$$GE = (NEm+NEa+NE_1+NEp)/REM/ DE /100, REM = 1.123$$
$$– (0.004092) * DE + 0.00001126 * (DE2 – 25.4/DE) \text{ and } CF * MBW$$

where gross energy (GE) was calculated with the (Equation 10.16) ignoring the net energy (NE) for grow (as they are grown animals and the NE for wool. The NE_1 = Milk * (1.47 + 0.4 * Kg Fat) and NEm is net energy for maintenance, NEa is net energy for activity, NE_1 is net energy for lactation, REM is ratio of net energy available in a diet for maintenance to digestible energy, DE is digestible energy as percentage of gross energy and CF is crude fat.

Madsen and Kjeldsen's formulas predicted CO_2 g/d, and this prediction was multiplied by the ratio of CH_4c to CO_2c to calculate CH_4p. Variance components were estimated with a repeatability animal model in ASReml v. 4.2.1. The model was as follows:

$$y = Xb + Z_aa + Z_ppe + e,$$

where y is the vector of phenotypes; b is the vector of fixed effects: herd, year-season interaction (n = 145), week of lactation (1–60), age of cow at calving in months (21–122) nested within parity (1,2, ≥ 3); a is the vector of direct additive genetic effects; pe the vector of random permanent environment effects; and e is the vector of residual effects. The matrices X, Z_a and Z_p are the incidence matrix relating observations with the fixed effects, random genetic effects, and random permanent environment effects, respectively. Distributions of the random effects are (1) $var(a) = A\sigma^2_a$ where A is the pedigree relationship matrix and σ^2_a is the animal additive genetic variance, and (2) $var(pe) = I\sigma^2_{pe}$, where I is an identity matrix of

an order equal to the number of observations and σ^2_{pe} is the permanent environmental variance., and (3) $var(e) = I\sigma^2_e$, where I is an identity matrix of an order equal to the number of observations and σ^2_e is the residual variance. Pedigree included 64,334 animals with on average 14 generations. Bivariate analyses were conducted to estimate the genetic correlations among the different phenotypes. Due to the imbalance in record numbers of animals and records between traits, estimates of some pairwise bivariate analyses may be less precise and have reported large standard errors. Therefore, genetic parameters involving these traits (Madsen and Kjeldsen 2) should be interpreted with caution.

Descriptive statistics for the different phenotypes are summarized in Table 1. The average CH_4c phenotypes were 401.8 ppm for conc, 1.5 for npeaks, and 954.1 ppm for speaks. The average CH_4p (g/d) varied depending on the formula, ranging from 189.0 ± 101.8 (Chagunda) to 355.1 ± 68.1 (Tier2). The average CH_4p (g/d) was 296.1 ± 132.0 for Madsen, 309.3 ± 135.1 for Kjeldsen2, and 318.5 ± 128.8 for Kjeldsen3, indicating that these formulas predicted similar values for CH_4p, which was expected as their formulas are the most similar. The average MeI was 9.9 g CH_4p per kg of MY.

Estimated variance components are presented in Table 2. Heritabilities for CH_4c phenotypes were 0.16 for conc, 0.29 for speaks, and 0.30 for npeaks. Heritabilities for average CH_4c have been widely reported in the literature, ranging between 0.11 and 0.18 (Lopez-Paredes et al., 2020; Manzanilla-Pech et al., 2020; van Breukelen et al., 2023). Unlike these studies, Reintke et al. (2020) was the only study reporting heritability for the number of eructation peaks, which was 0.02 with a standard error (SE) of 0.05. This study was conducted in ewes, and the lower heritability may be due to the method (laser methane detector) rather than the phenotype. Heritabilities for CH_4p ranged between 0.03 (Madsen) and 0.27 (Tier2). The heritabilities for the ratio and MeI were 0.08, and was 0.13 for CO_2c. Larger heritabilities (0.11–0.47) have been reported for CH_4p using the Madsen formula by Manzanilla et al. (2022a, 2023) in a Danish Holstein population and by Sypniewski et al. (2021) in a Polish Holstein population. Genetic correlations between CH_4c phenotypes (Figure 1) ranged from moderate between avg and npeaks (0.49, SE = 0.06) to highly positive between avg and speaks (0.85, SE = 0.02). As expected, the number of eructation peaks is moderately correlated with the 2 other CH_4c phenotypes. However, since it does not reflect the intensity of the eructation,

Table 1. Descriptive statistics for the CH_4c per visit (avg), sum of maximum peaks per minute (speaks), number of peaks per minute (npeaks), CH_4p calculated by Madsen, Chagunda, Kjeldsen2, Kjeldsen3 and Tier2, ratio between CH_4c and CO_2c, carbon dioxide per visit (CO_2c), methane intensity (MeI), milk yield (MY) and body weight (BW)

Type	Trait	#cows	#records	mean	SD	CV (%)
CH_4c (ppm)	avg	7,110	114,574	401.8	179.1	44
	npeaks	7,129	114,744	1.5	0.4	26
	speaks	7,018	114,244	954.1	458.9	48
CH_4p (g/d)	Madsen	806	10,668	296.1	132.0	44
	Chagunda	868	16,822	189.0	101.8	54
	Kjeldsen2	798	10,676	309.3	135.1	44
	Kjeldsen3	6,608	73,799	318.5	128.8	40
	Tier2	855	14,635	355.1	68.1	19
	ratio	6,945	99,931	0.07	0.02	28
	CO_2c	7,529	141,591	5,376.0	1,846.0	34
Intensity (g CH_4p /kg MY)	MeI	6,608	73,799	9.9	4.8	48
	MY (kg/d)	6,774	84,287	34.4	9.6	28
Production traits	BW (kg)	868	16,868	697.6	82.7	12

Table 2. Estimated genetic variance ($\sigma_2 a$), phenotypic variance ($\sigma_2 p$), heritability (h^2) and repeatability (rep) for the average $CH_4 c$ per visit (avg), sum of maximum peaks per minute (speaks), number of peaks per minute (npeaks), $CH_4 p$ calculated by Madsen, Chagunda, Kjeldsen2, Kjeldsen3 and Tier2; carbon dioxide concentration per visit ($CO_2 c$), milk yield (MY), methane intensity (MeI) and body weight (BW)

Trait	$\sigma_2 a$	$\sigma_2 pe$	$\sigma_2 p$	h^2	rep
avg	3540.4	6049.40	21979.0	0.16 (0.02)	0.43 (0.01)
speaks	49496.8	37027.6	171040.0	0.29 (0.02)	0.51 (0.02)
npeaks	567.7	425.558	1889.2	0.30 (0.02)	0.52 (0.02)
Madsen	245.1	2585.2	8369.8	0.03 (0.03)	0.34 (0.03)
Chagunda	1670.0	2548.9	7914.0	0.21 (0.06)	0.53 (0.05)
Kjeldsen2	224.1	2377.39	8062.0	0.03 (0.03)	0.32 (0.03)
Kjeldsen3	707.6	1504.6	8465.7	0.08 (0.01)	0.26 (0.01)
Tier2	704.8	1066.2	2608.7	0.27 (0.07)	0.68 (0.06)
ratio	0.28	0.46	3.53	0.08 (0.01)	0.21 (0.01)
$CO_2 c$	2649.1	6448.6	20725.0	0.13 (0.02)	0.44 (0.01)
MY	12.2	22.1	54.6	0.22 (0.02)	0.62 (0.02)
MeI	1.1	3.1	13.5	0.08 (0.01)	0.31 (0.01)
BW	2109.2	1313.2	3760.1	0.56 (0.08)	0.91 (0.08)

only the number, it is not a good candidate for a breeding goal trait. Conversely, speaks is highly correlated with conc, meaning that an animal with a high $CH_4 c$ during the entire period also has a high value during the eructation peaks. Genetic correlations between $CH_4 p$ phenotypes were highly positive (0.89 to 1.0), except for Tier2, indicating that despite numerical differences in the mean values, they rank the animals similarly (high and low emitters). Tier2 presented low to moderate (0.04 to 0.37) genetic correlations with the other $CH_4 p$ phenotypes. Tier2 is used more as a control, as it does not involve any input from the sniffers but relies entirely on MY and BW. It has been developed to predict $CH_4 p$ from a group of animals rather than individually (IPCC, 2006; 2019). However, it is important to mention that due to the low number of animals for some of the formulas that include BW in their calculation, some of the SEs were high (0.06 to 0.30). Currently, there is no published information on the genetic correlations between the methane phenotypes presented in this study for either $CH_4 c$ or $CH_4 p$, except for avg and $CH_4 p$ with Madsen formula. Genetic correlations between avg and $CH_4 p$ were high (0.77–0.93), except for Tier2 (−0.08). Genetic correlations between speaks and $CH_4 p$ were positive, ranging from 0.36 (Kjeldsen2) to 0.95 (Chagunda), and negative for Tier2 (−0.19). Similarly, npeaks correlations were positive, ranging from 0.52 (Kjeldsen3) to 0.99 (Kjeldsen2), and negative for Tier2 (−0.39). Genetic correlations between ratio and $CH_4 c$ ranged from low negative (−0.18 to −0.08; speaks and conc) to moderate positive (0.59; npeaks). In contrast, the majority of genetic correlations between $CH_4 p$ phenotypes and ratio were positive, ranging from 0.35 (Madsen) to 0.98 (Kjeldsen3), except for Tier2 (−0.25). No genetic correlation was estimated between $CO_2 c$ and npeaks; however, moderate positive genetic correlations from 0.49 ($CO_2 c$-speaks) to high correlations of 0.84 ($CO_2 c$ and avg) were estimated. Genetic correlations between $CO_2 c$ and $CH_4 p$ phenotypes were mostly moderate positive (0.18–0.58), except for Kjeldsen2 (−0.73) with large SE (0.17).

Methane intensity presented the largest correlations with avg (0.77) and the smallest with npeaks (0.29), whereas for $CH_4 p$ phenotypes, the largest positive correlation was with Madsen (0.57) and the smallest with Kjeldsen2 (0.03). However, as $CH_4 p$ itself is derived from ECM and/or BW, mathematical dependencies may artificially affect genetic correlations with MeI. Further, the genetic correlation between MeI and Tier2 was the largest negative (−0.90), which may also be affected by the shared use of MY in the calculation of both traits. Genetic correlations between MY and avg (0.30) and between MY and speaks (0.46) were moderately positive, whereas with npeaks it was close to zero. This means that $CH_4 c$ phenotypes such as avg and speaks are not entirely independent of milk. Most of the genetic correlations between MY and $CH_4 p$ were positive (0.32–0.94), except for Kjeldsen2 (−0.69) with large SE (0.97).

Finally, genetic correlations between BW and all $CH_4 c$ phenotypes were close to zero, meaning that body weight is independent of $CH_4 c$, as expected. For $CH_4 p$, the genetic correlations with BW were negative for Madsen (−0.30) and Kjeldsen2 (−0.72) and Kjeldsen3 (−0.23), which can be explained by the indirect use of BW (in combination with ECM) in the prediction of CO_2 for Madsen and Kjeldsen2. Positive genetic correlations between BW and Chagunda (0.36) and Tier2 (0.57) could be explained by the use of BW in the calculation of $CH_4 p$. Unlike the previously reported positive genetic correlation (0.65) between MeI and BW by Manzanilla-Pech et al. (2021) in an international data set, in this study, this correlation was moderately negative (−0.45). One disadvantage of $CH_4 p$ phenotypes using the formulas and MeI is the artificially created dependency on other traits that are used for their calculation, such as ECM and BW.

All phenotypes exhibited varying degrees of heritability. Average $CH_4 c$ showed consistently high positive genetic correlations with all other methane phenotypes except for ratio and Tier 2 (benchmark). The $CH_4 p$ phenotypes calculated using the formulas also presented strong positive correlations among themselves and with other methane traits. Given these results, avg and speaks appear to be good proxies for methane emissions, especially as they showed minimal correlation with body weight and no induced dependencies as the $CH_4 p$ based on formulas. Overall, selecting for low-emitting animals is possible, regardless of the phenotype selected. The aim of this study is not to propose selection indicators, but rather to provide a fair comparison of different methane phenotypes that could potentially be used in future selection strategies for lower methane emissions when recording with sniffers. In this context, methane concentration is considered the reference trait for methane emissions.

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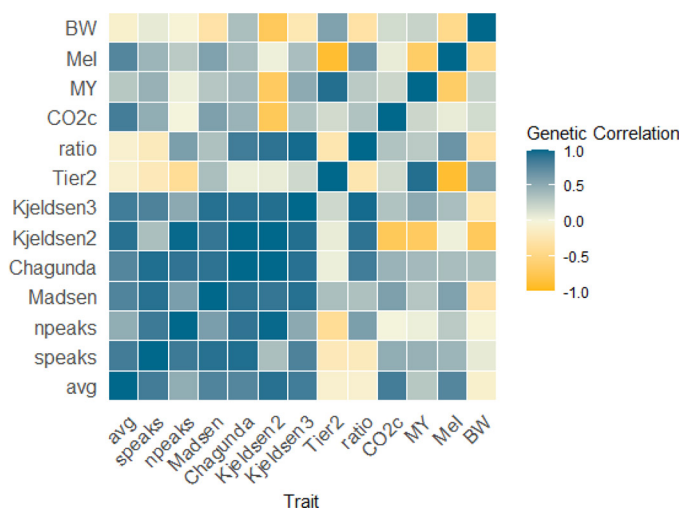
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Figure 1. Genetic correlations among the CH_4c per visit (avg), sum of max peaks per minute (speaks), number of peaks per minute (npeaks), CH_4p calculated by Madsen, Chagunda, Kjeldsen2, Kjeldsen3 and Tier2, carbon dioxide concentration per visit (CO_2c), milk yield (MY), methane intensity (MeI) and body weight (BW).