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Predicting CO_2 production of lactating dairy cows from animal, dietary, and production traits using an international dataset

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ABSTRACT

Automated measurements of the ratio of concentrations of methane and carbon dioxide, $[CH_4]:[CO_2]$, in breath from individual animals (the so-called “sniffer technique”) and estimated CO_2 production can be used to estimate CH_4 production, provided that CO_2 production can be reliably calculated. This would allow CH_4 production from individual cows to be estimated in large cohorts of cows, whereby ranking of cows according to their CH_4 production might become possible and their values could be used for breeding of low CH_4-emitting animals. Estimates of CO_2 production are typically based on predictions of heat production, which can be calculated from body weight (BW), energy-corrected milk yield, and days of pregnancy. The objectives of the present study were to develop predictions of CO_2 production directly from milk production, dietary, and animal variables, and furthermore to develop different models to be used for different scenarios, depending on available data. An international dataset with 2,244 records from individual lactating cows including CO_2 production and associated traits, as dry matter intake (DMI), diet composition, BW, milk production and composition, days in milk, and days pregnant, was compiled to constitute the training dataset. Research location and experiment nested within research location were included as random intercepts. The method of CO_2 production measurement (respiration chamber [RC] or GreenFeed [GF]) was confounded with research location, and therefore excluded from the model. In total, 3 models were developed based on the current training dataset: model 1 (“best model”), where all significant traits were included; model 2 (“on-farm model”), where DMI was excluded; and model 3 (“reduced on-farm model”), where both DMI and BW were excluded. Evaluation on test dat sets with either RC data (n = 103), GF data without additives (n = 478), or GF data only including observations where nitrate, 3-nitrooxypropanol (3-NOP), or a combination of nitrate and 3-NOP were fed to the cows (GF+: n = 295), showed good precision of the 3 models, illustrated by low slope bias both in absolute values (−0.22 to 0.097) and in percentage (0.049 to 4.89) of mean square error (MSE). However, the mean bias (MB) indicated systematic overprediction and underprediction of CO_2 production when the models were evaluated on the GF and the RC test datasets, respectively. To address this bias, the 3 models were evaluated on a modified test dataset, where the CO_2 production (g/d) was adjusted by subtracting (where measurements were obtained by RC) or adding absolute MB (where measurements were obtained by GF) from evaluation of the specific model on RC, GF, and GF+ test datasets. With this modification, the absolute values of MB and MB as percentage of MSE became negligible. In

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

conclusion, the 3 models were precise in predicting CO_2 production from lactating dairy cows.

Key words: tracer gas, cattle, heat production, model evaluation

INTRODUCTION

Quantification of enteric methane (CH_4) production is increasingly important, as it is required to evaluate CH_4 mitigation strategies in greenhouse gas inventories and for calculating the carbon footprint of the beef and dairy industry. However, large-scale direct measurement of CH_4 with respiration chambers (**RC**), GreenFeed head chambers (**GF**), or the sulfur hexafluoride method (SF_6) is difficult, labor intensive, and costly. To address these challenges, models for predicting CH_4 production in cows fed specific diets have been developed (Appuhamy et al., 2016; Niu et al., 2018), although between-animal variation of CH_4 emission is ignored. Furthermore, CH_4-reducing feed additives are foreseen to be implemented in farm practice in the near future, and therefore prediction of CH_4 will require models that account for the effect of different additives, which requires a comprehensive dataset.

The sniffer technique is an alternative approach to estimate individual enteric CH_4 production in large-scale settings (Madsen et al., 2010; Lassen et al., 2012), and it offers an economically favorable alternative compared with other methods (RC, GF, and SF_6). Installation of the sniffer equipment in combination with a concentrate bin will allow measurements of the ratio between concentration of CH_4 and concentration of carbon dioxide (CO_2) in breath exhaled by the cows, when they visit the bin (Madsen et al., 2010). Compared with the approach of predicting CH_4 production by a model, between-animal variation is accounted for by the sniffer technique, as the CO_2 production is calculated and used to estimate the individual CH_4 production based on the ratio of $[CH_4]$:$[CO_2]$ in exhaled breath. The sniffer technique as such is therefore not a quantitative measure of emissions, like RC and GF, but it relies on calculating CH_4 emission by combining the predicted CO_2 production with gas concentration ratio measured by use of a given instrument. The idea is that CO_2 production is more accurately predicted from animal, dietary, and production traits than CH_4. Therefore, CO_2 production from dairy cows can be estimated as in Pedersen et al. (2008) and Madsen et al. (2010) in Equations [1] and [2], respectively:

$$CO_2\ (\text{L/d}) = \text{HPU/d} \times 180\ \text{L}\ CO_2\text{/h/HPU} \times 24\ \text{h}, \quad [1]$$

where heat-producing units (**HPU**) are equal to the heat production (**HP**) of an animal (when expressed in W/d) divided by 1,000 W; and

$$CO_2\ (\text{L/d}) = \text{HP (kJ/d)}/21.75\ \text{kJ/L}\ CO_2, \quad [2]$$

where 21.75 kJ is an estimate of HP when 1 L of CO_2 is exhaled due to the metabolism of nutrients of an average cow diet (Chwalibog, 1991). Furthermore, because the unit of HP in Equation [3] is W/d, and 1 W = 1 J/s, therefore HP (kJ/d) = (HP (W/d) × 60 s × 60 min × 24 h)/1,000.

One of the equations currently used to estimate HP is based on metabolic BW (**$BW^{0.75}$**), ECM (kg/d; Sjaunja et al., 1990), and days in pregnancy (**DIP**), and it originates from a report by Commission Internationale du Génie Rural (CIGR, 2002), where the following model was developed to quantify needed barn ventilation on group level of dairy cows:

$$\text{Heat production (W/d)} = 5.6 \times \text{BW}\ (\text{kg}^{0.75}) + 22 \times \text{ECM (kg/d)} + 1.6 \times 10^{-5} \times \text{DIP}^3. \quad [3]$$

Measuring the CH_4 production from cows in large-scale settings plays a crucial role in identifying low CH_4-emitting cows, forming the basis for genetic selection aimed at reducing CH_4 emission. This approach was used by Manzanilla-Pech et al. (2022), where sniffer data created the basis for calculating genetic correlations between CH_4 traits and other phenotypes. The approach of using CO_2 as an internal marker has also been used to predict ammonia emissions at barn level (Kai et al., 2017). Measuring gas emissions by RC is considered "the gold standard," but although the CH_4 and CO_2 production as such is not measured with the sniffer method, measured CH_4 concentration values by the sniffer method are well correlated (r = 0.75, based on random cow effects) with data obtained in RC (Difford et al., 2019). However, based on a minor Danish dataset and using Equation [3] in combination with Equation [1] on data from RC, Hellwing et al. (2013) concluded on a dataset, which is now a minor part of the current training dataset from which the models are derived, that the sniffer method underestimated the actual production of CO_2 and thereby the production of CH_4 as well. A part of the explanation lays in the use of HP as an intermediate step to calculate CO_2 production, as HP is dependent on the energy balance of the cow (Huhtanen et al., 2020) and nutrient composition of the diet (Kirchgessner and Muller, 1998). The sniffer method only measures the concentration of CO_2 and CH_4, and, to estimate CH_4 emission from cattle, it relies on a prediction equation for CO_2 production. We hypothesized that CO_2 production can be predicted directly from dietary variables, milk production, and animal variables. The objectives were to (1) identify variables that explain variance in CO_2 production from dairy cows, (2) develop a CO_2 prediction model with the most determining variables, ignoring that some variables may be difficult to obtain on farms, and (3) develop models that can be applied on commercial farms to estimate CO_2 production from dairy cows.

MATERIALS AND METHODS

Dataset

Members of the Feed and Nutrition Network of the Global Research Alliance on Agriculture Greenhouse Gases (FNN, 2023) provided data for the present study. As some research groups have more than 1 experimental location, the dataset contains data from 12 research groups, covering 15 different locations in North America, Europe, and Oceania, derived from 76 experiments conducted from 1989 until 2019 (Tables 1 and 2). Some limitations for inclusion of data were predefined: (1) to ensure data quality, measurement of gas exchange should have been performed either in RC or with GF (C-Lock Inc., Rapid City, SD); (2) only data from lactating dairy cows were included; (3) records of CO_2 production should be available; and (4) data had to be on an individual animal level. The initial dataset contained 3,179 individual animal records. Because BW, ECM yield, and DIP were data used to predict HP (CIGR, 1984), they were considered as being highly important in the present study as well due to their expected correlation to CO_2 production, but not all datasets included DIP.

Data Pre-Processing

Data pre-processing was necessary before model development to cope with incomplete and inconsistent records, or use of different units for a given variable. Records based on a diet containing monensin were excluded (n = 23) given its noncompliance with EU regulations. Despite the feed additives 3-nitrooxypropanol (**3-NOP**) and nitrate not being used in all countries at present, records were kept in the training dataset if these specific additives were supplied to the cows. Records with missing values for CO_2 (n = 192) production were also excluded. Furthermore, records from cows with more than 300 DIM (n = 140) were excluded, as they constitute a small group of animals in the dataset, which is not representative for a commercial farm. After this selection, the pre-training dataset contained n = 2,824 records.

Records were categorized into 4 breed groups, (1) Holstein, (2) Jersey, (3) Ayrshire, or (4) other breeds and crossbreeds, and 3 parity groups: (1) first, (2) second, or (3) third parity and higher. Emissions of CO_2 were reported as grams per day (g/d) or liters per day (L/d). If the research locations delivered the measured gas exchange in liters per day, the ideal gas law was used

Table 1. Overview of each research location in the refined training dataset (n = 2,244) after data pre-processing

Research location	n	Experiments (n)	Gas measuring method (n)	Breeds (n)	Parity of lactating cows (n)
Aarhus University	313	18	Respiration chamber	Holstein (271), Jersey (42)	First (118), second (112), third and older (83)
AgResearch Lincoln	28	1	GreenFeed	Others/crossbreeds	First (3), second (6), third and older (19)
AgResearch Palmerston North	56	5	Respiration chamber	Others/crossbreeds	Second (9), third and older (47)
Agriculture Victoria Research	196	7	Respiration chamber	Holstein	First (23), second (52), third and older (121)
ETH Zurich	41	2	Respiration chamber	Holstein (7), others/crossbreeds (34)	First (4), second (7), third and older (30)
Flanders Research Institute for Agriculture, Fisheries and Food	141	8	Respiration chamber	Holstein	First (27), second (56), third and older (58)
INRAE	118	7	Respiration chamber	Holstein	First (17), second (56), third and older (45)
PennState	418	8	GreenFeed	Holstein	First (148), second (147), third and older (123)
Research Institute for Farm Animal Biology	19	1	Respiration chamber	Holstein	Second (10), third and older (9)
Swedish University of Agricultural Sciences	455	3	GreenFeed	Ayrshire (96), others/crossbreeds (359)	First (167), second (128), third and older (160)
TU Munich	51	1	Respiration chamber	Others/crossbreeds	First (3), second (17), third and older (31)
USDA Beltsville Agricultural Research Center	45	1	Respiration chamber	Holstein (22), Jersey (23)	Second (22), third and older (23)
University of Milan	62	3	Respiration chamber	Holstein	First (18), second (33), third and older (11)
University of Reading	106	6	Respiration chamber	Holstein	Second (25), third and older (81)
Wageningen University and Research	195	5	Respiration chamber	Holstein	First (52), second (55), third and older (88)
All	2,244	76	Respiration chamber (1,343) GreenFeed (901)	Ayrshire (96), Holstein (1,555), Jersey (65), others/crossbreeds (528)	First (580), second (735), third and older (929)

Table 2. Summary statistics of the continuous parameters included in the training dataset (n = 2,244)

Item	n	Mean	SD	Minimum	Maximum
Dietary composition					
OM (g/kg DM)	2,227	924	17.0	833	953
CP (g/kg DM)	2,244	167	22.6	81.0	253
CF (g crude fat/kg DM)	2,244	38.8	11.22	12.1	74.0
DMI (kg/d)	2,244	20.7	4.49	6.80	37.2
Milk (kg/d)	2,244	30.1	9.09	2.65	65.7
ECM (kg/d)	2,244	30.6	8.35	2.91	71.5
Milk composition					
CF (g crude fat/kg)	2,244	42.7	8.74	13.2	88.5
CP (g/kg)	2,244	33.3	3.92	23.0	53.9
Lactose (g/kg)	2,148	47.7	2.87	26.0	56.3
Days in pregnancy (d)	562	56	60.0	0	233
DIM (d)	2,244	137	69.2	7	299
BW (kg)	2,244	606	82.0	341	969
CO_2 (g/d)	2,244	12,402	2,023.0	4,937	20,950
CH_4 (g/d)	2,241	397	85.8	136	729

to convert to grams per day, with the conversion factor depending on the temperature and pressure at the specific research location. The outcome of the models is CO_2 in grams per day at standard temperature and pressure (0°C and 101.325 kPa). If needed, the outcome of the model can be converted to liters per day as CO_2 (L/d) = CO_2 (g/d) × 0.509 (L/g). Yield of ECM (3.14 MJ/kg) was calculated according to the respective ECM equations in Sjaunja et al. (1990) based on fat, protein, and lactose concentration, taking into account lactose reported as monohydrate or in anhydrous form (15.71 kJ/g and 16.54 kJ/g, respectively). Conversion from true protein to CP was performed with the factor 1.058 (DePeters and Ferguson, 1992).

Model Development

Individual DMI is often available at research facilities but not on commercial farms. In addition, only some commercial farms continuously track cows' BW. Due to the difference in data availability in different settings, 3 models were developed to cover these different scenarios. Before the continuous predictor variables were included in the model development, Pearson correlation coefficients (r) were calculated (Supplemental Table S1, see Notes). In case of 2 variables being highly correlated ($r \geq 0.5$), only the predictor variable with the highest correlation coefficient to CO_2 production was chosen. This, for instance, excluded milk production (kg/d) and ECM (kg/d) from being predictor variables in the same model (r = 0.93). They were equally correlated with CO_2 production (both r = 0.50), and ECM was chosen for model development, as inclusion of milk production led to a high number of interactions between milk production and milk nutrients (data not shown). Also, DMI and ECM were highly correlated (r = 0.75), where DMI had the strongest correlation with CO_2 production (r = 0.69); therefore they could not be predictor variables in the same model. Furthermore, milk crude fat (**CF**; g/kg) and milk CP (g/kg) were highly correlated (r = 0.54). Therefore, only milk CF was included in the model development, since its correlation to CO_2 production was stronger (r = −0.19) than it was for milk CP (r = −0.09). Based on the described exclusion of predictor variables, continuous predictor variables were DMI, ECM yield, concentrations of fat and lactose in milk, $BW^{0.75}$, DIM, DIP, and dietary CP and CF concentration. Parity and breed were set as discrete factors. Breed was only to some extent confounded to research location (Table 1); therefore it was per default included in all models as a fixed effect. Also, research location and experiment nested within research location were per default included as random effects (allowing individual intercepts) in all 3 models. Method of measuring CO_2 production (RC or GF) was confounded with research location, and none of the research locations provided data obtained by both GF and RC. Therefore, measurement method was not included as a predictor variable by itself, as it was indirectly included through the random effect of research location. As some of the variables by nature have different units (e.g., DIM and ECM), all values were centered (mean = 0) by using the "scale" function in R (R Core Team, 2023). No standardization was performed (original variation was kept) to ease the implementation of the models. All statistical analyses were conducted in R 4.3.0 (R Core Team, 2023). The selection of each model was performed with the buildmer function (Voeten, 2023), using the default criterion likelihood ratio test for selection of predictor variables. Using Akaike's information criterion or Bayesian information criterion as criteria instead of likelihood ratio test resulted in the selection of the same variables, regardless of whether the stepwise inclusion or elimination followed a "forward" or "backward" direction order. The 3 basic models derived from the buildmer function were tested for increased complexity by adding interactions and afterward testing for inclusion of a random slope of one of the predictor variables. Analysis of variance tests were performed to determine the level of significance of increased model complexity (fitting with either "ML" or "REML," depending on the 2 models compared). Statistical significance was declared at $P \leq 0.05$. Based on the significant predictor variables from the model development, a common training dataset (n = 2,259), without missing records for DMI, DIM, ECM, BW, dietary CP, dietary CF, milk CF, parity, and breed was used to derive all 3 models. Another approach would have been to train different models using different datasets, containing the predictor variables of interest to maximize the number of records (Niu et al., 2018). However, to perform unbiased comparisons across models, the same dataset was used for all models in the current study. Outliers, here defined as records with residuals >5 × SD (residuals derived with model 1, n = 15), were removed from the dataset. The residuals of each model were plotted against predicted values of CO_2 production and against individual variables. The residuals did not show any nonlinear relationship; therefore data were not transformed. The refined training dataset contained 2,244 individual animal records (Tables 1 and 2), where 60% of the records were obtained by RC.

Model Evaluation

Model performance was tested on 3 datasets from Aarhus University, including (1) solely RC data (without any CH_4-reducing feed additives), (2) solely GF data (without any CH_4-reducing feed additives), and (3) GF data with observations where only nitrate, only 3-NOP, or both nitrate and 3-NOP were fed (Table 3). All test datasets consisted of data obtained in studies performed after the current training dataset was collected (from 2020 to 2022), and the training dataset was tested again with different applicable models as shown in Table 4.

The RC test dataset (n = 103) consisted of data from 5 studies; 4 Latin square designs and 1 crossover design, and records having DIM >300 d were excluded from the test dataset. All animals were Holstein cows and were 136 ± 64.4 DIM (±SD), with a DMI of 21.4 ± 3.76 kg/d and an ECM of 31.3 ± 6.92 kg/d. The percentages of cows in first, second, and third and higher lactation in the dataset were 39%, 47%, and 15%, respectively.

The GF test dataset (n = 478) consisted of data from a part of 4 production trials; 3 Latin square designs and 1 continuous trial were included, and in case of the latter, an average from the last week of measuring was included. Records having DIM >300 d were excluded. All animals were Holstein cows and were 145 ± 52.0 DIM, with a DMI of 21.3 ± 3.06 kg/d and yielding 33.8 ± 6.56 kg ECM/d. The percentages of first-, second-, and third-lactation and older cows in the dataset were 50%, 27%, and 23%, respectively.

The last test dataset consisted only of records where the additives nitrate and 3-NOP were supplemented (GF+, n = 295). The data were from a part of 2 production trials, which were both Latin square designs. None of the records had DIM >300 d. All animals were Holstein cows and were 107 ± 43.6 DIM, with a DMI of 20.0 ± 3.21 kg/d and an ECM of 31.2 ± 6.14 kg/d. The percentages of cows in first, second, and third and higher lactation in the dataset were 52%, 25%, and 23%, respectively.

In addition, the models were also evaluated on a modified and merged version of the 3 test datasets (RC, GF, and GF+; Table 5) and on the training dataset (Table 6). In the modified dataset, the records of the measured CO_2 production (g/d) from the test datasets subtracted (where measurements were obtained by RC) or added (where measurements were obtained by GF) the absolute mean bias (**MB**, here calculated as observed − predicted), from the model evaluation of the specific model on RC, GF, and GF+.

The "opmetrics" function from the R package modMetricsR (Giagnoni, 2023) was used to obtain the evaluation estimates: root mean square error (**RMSE**), RMSE as percentage of observed mean, mean absolute error

Table 3. Summary statistics of the continuous parameters and CO_2 production (g/d) in the test dataset obtained from respiration chambers (RC, without additives, n = 103), GreenFeed (GF, without additives test dataset, n = 478), or GreenFeed only including diets containing nitrate, 3-nitrooxypropanol, or both nitrate and 3-nitrooxypropanol (GF+, n = 295); all data were obtained at Aarhus University (Viborg, Denmark) from 2020 to 2022

	Mean			SD			Minimum			Maximum		
Test dataset	RC[1]	GF[2]	GF+[3]	RC	GF	GF+	RC	GF	GF+	RC	GF	GF+
DMI (kg/d)	21.4	21.3	20.0	3.76	3.06	3.21	11.8	13.9	11.7	27.4	30.0	27.6
Diet CP (g/kg DM)	171	165	171	7.9	8.4	11.3	157	149	148	188	188	186
CF (g crude fat/kg DM)	33.9	44.1	41.7	8.52	13.3	15.6	23.7	27.2	26.3	62.0	70.6	69.1
ECM (kg/d)	31.3	33.8	31.2	6.92	6.56	6.14	17.4	16.1	17.4	47.4	54.3	49.6
Milk CF (g crude fat/kg)	39.3	39.4	40.9	6.75	6.25	5.84	23.4	18.6	23.1	58.9	58.7	57.7
DIM (d)	136	145	107	64.4	52.0	43.6	42	16	24	297	290	231
BW (kg)	640	655	642	53.1	67.6	62.8	550	500	496	747	873	858
CO_2 (g/d)	14,369	12,625	12,163	1,663.8	1,531.0	1,468.0	11,086	8,617	8,213	18,215	17,782	15,365

[1]The percentages of cows in first, second, and third and higher lactation in the dataset were 39%, 47%, and 15%, respectively. All cows were Holstein cows.

[2]The percentages of first, second, and third and older cows in the dataset were 50%, 27%, and 23%, respectively. All cows were Holstein cows.

[3]The percentages of cows in first, second, and third and higher lactation in the dataset were 52%, 25%, and 23%, respectively. All cows were Holstein cows.

Table 4. Coefficients of the 3 models to predict CO_2 (g/d) from lactating dairy cows, where model 1 is "best model," model 2 is "on-farm model," and model 3 is "reduced on-farm model"[1]

Item	Model 1	Model 2	Model 3
Intercept	956	−6,134	8,781
DMI (kg/d)	122		
ECM (kg/d)		213	80.3
MetaBW (kg)	60.4	126	
Diet CP (g/kg DM)	3.44		
Milk CF (g/kg)		52.5	
DIM (d)		−5.13	−4.66
Breed			
Ayrshire	0	0	0
Holstein	−777	2,117	−49.0
Jersey	1,103	1,364	−2,321
Others/crossbreeds	1,501	4,083	−1,237
Parity			
First			0
Second			511
Third and higher			1,587
DIM × Diet CF		- 0.122	−0.149
ECM × DIM		0.386	0.338
ECM × metaBW		−1.18	
Milk CF × metaBW		−0.614	
DMI × Ayrshire	0		
DMI × Holstein	206		
DMI × Jersey	204		
DMI × others/crossbreds	225		
DMI × first parity	0		
DMI × second parity	7.53		
DMI × third parity	15.7		
MetaBW × Ayrshire	0	0	
MetaBW × Holstein	−18.5	−5.96	
MetaBW × Jersey	−37.3	−1.03	
MetaBW × others/crossbreds	−43.2	−33.4	
DIM × Ayrshire		0	0
DIM × Holstein		2.06	6.05
DIM × Jersey		2.49	6.02
DIM × others/crossbreds		8.94	11.3
MetaBW × first parity		0	
MetaBW × second parity		3.66	
MetaBW × third parity		4.01	
First parity × milk CF			−4.18
Second parity × milk CF			−10.5
Third parity × milk CF			−28.8
Ayrshire × first parity			0
Ayrshire × second parity			0
Ayrshire × third parity			0
Holstein × first parity			0
Holstein × second parity			775
Holstein × third parity			803
Jersey × first parity			0
Jersey × second parity			608
Jersey × third parity			1,307
Others/crossbreds × first parity			0
Others/crossbreds × second parity			791
Others/crossbreds × third parity			659

[1]Diet CF = dietary crude fat (g/kg DM), diet CP = dietary crude protein (g/kg DM), DIM = days in milk (d), DMI = dry matter intake (kg/d), ECM = energy-corrected milk yield (kg/d), milk CF = milk crude fat (g/kg), metaBW = metabolic body weight = body weight$^{0.75}$ (kg).

(**MAE**), concordance correlation coefficient (**CCC**), ratio of RMSE to standard deviation of measured data (**RSR**), MB, slope bias (**SB**), and MB, SB, and dispersion as percentage of mean square error (**MSE**). Both CCC and RSR are dimensionless parameters. The CCC is the product of Pearson correlation coefficient (r; ranging from −1 to +1) and the bias correction factor (C_b; ranging from 0 to 1). Perfect fit (precision) is indicated by r = 1, and agreement between predicted and observed values (accuracy) is indicated by C_b = 1 and thus CCC = 1.

RESULTS

Due to the incorporation of data from different research locations in the training dataset, a high level of variability was present (Table 2). The DMI ranged from 6.80 to 37.2 kg/d, and CH_4 and CO_2 production varied from 136 to 729 g/d and 4,937 to 20,950 g/d, respectively.

Description of Models 1, 2, and 3

The different models developed to be used in different practical settings, depending on data availability, were as follows.

Model 1, intended to be used in a situation where individual DMI data are available ("best model"; e.g., at research locations), and where DMI alone described 58% of the variation in CO_2 production (g/d) in the present dataset, reads,

$$\textbf{Model 1} \text{ ("best model"): } CO_2\ (g/d) = b_0 + (b_1 \times DMI) + (b_2 \times BW^{0.75}) + (b_3 \times \text{Diet CP}) + \text{breed} + (b_{DMI,breed} \times DMI) + (b_{DMI,parity} \times DMI) + (b_{BW^{0.75},breed} \times BW^{0.75}),$$

where, b_0 is the intercept; b_1, b_2, and b_3 are the coefficients of DMI (kg/d), $BW^{0.75}$ ($kg^{0.75}$), and diet CP (g/kg DM), respectively; and $b_{DMI,breed}$ and $b_{BW^{0.75},breed}$ are the breed-specific coefficients of DMI and $BW^{0.75}$. The parity-specific coefficient of DMI is $b_{DMI,parity}$. All coefficients are listed in Table 4.

An example of using model 1 to calculate the CO_2 production (g/d) from a second-parity Holstein cow, with a DMI of 25 kg DM/d, with 160 g dietary CP per kilogram DM, weighing 600 kg, is as follows:

$$956 + [122 \times 25\ (kg\ DM/d)] + [60.4 \times 600\ (kg^{0.75})] + [3.44 \times 160\ (g\ CP/kg\ DM)] - 777 + [206 \times 25\ (kg\ DM/d)] + [7.53 \times 25\ (kg\ DM/d)] + [-18.5 \times 600\ (kg^{0.75})] = 14{,}197\ g\ CO_2 \text{ per day.}$$

Model 2 was intended for an on-farm setting, where individual DMI data are not available ("on-farm model"); therefore ECM became a significant predictor variable, as ECM alone described 28% of the variation in CO_2 production (g/d) in the present dataset. It reads,

Table 5. Model evaluation of the 3 models to predict CO_2 production (g/d) from lactating dairy cows, where model 1 is "best model," model 2 is "on-farm model," and model 3 is "reduced on-farm model"[1]

Item	Test dataset	RMSE	RMSE, % mean	MAE	CCC	RSR	MB	SB	MB, % MSE	SB, % MSE	Dispersion, % MSE
Model performance[2]											
Model 1	RC	1,456	10.1	1,281	0.66	0.88	1,134	−0.065	60.6	0.44	38.9
	GF	1,046	8.29	856	0.76	0.68	−655	−0.040	39.1	0.27	60.6
	GF+	949	7.81	756	0.79	0.65	−587	−0.052	38.2	0.54	61.3
Model 2	RC	1,416	9.85	1,240	0.66	0.85	1,192	0.097	70.9	0.85	28.2
	GF	1,187	9.40	993	0.67	0.78	−740	0.059	38.9	0.32	60.8
	GF+	1,199	9.86	959	0.63	0.82	−639	−0.063	28.4	0.35	71.3
Model 3	RC	1,847	12.9	1,635	0.54	1.11	1,619	−0.028	76.9	0.049	23.1
	GF	1,138	9.01	916	0.68	0.74	−138	−0.19	1.47	4.89	93.6
	GF+	1,172	9.64	931	0.61	0.80	−77.4	−0.22	0.44	4.68	94.9
Model performance[3]											
Model 1	Modified	806	6.15	616	0.85	0.52	0.27	−0.046	0.000	0.63	99.4
Model 2	Modified	941	7.15	742	0.77	0.61	−0.11	0.025	0.000	0.10	99.9
Model 3	Modified	1,118	8.88	886	0.69	0.72	0.024	−0.17	0.000	3.87	96.1

[1]Model performance was evaluated based on either of the following. (1) Observations from 3 test datasets (both from Aarhus University, Viborg, Denmark) obtained from respiration chambers (RC, n = 103) or GreenFeed units (GF, n = 478) without additives, or GF only including diets containing nitrate, 3-nitrooxypropanol, or both nitrate and 3-nitrooxypropanol (GF+, n = 295). Or (2) a modified dat set, with RC, GF, and GF+ test datasets merged together (n = 876). The CO_2 production (g/d) in the modified dataset was calculated by subtracting mean bias (if measurements were obtained by RC) or adding mean bias (if measurements were obtained by GF and GF+) from evaluation of the specific model on RC, GF, and GF+ test datasets. RMSE = root mean square error, MAE = mean absolute error, CCC = concordance correlation coefficient, RSR = ratio of RMSE to standard deviation of measured data, MB = mean bias, SB = slope bias, and MSE = mean square error.

[2]Test dataset based on observed CO_2 production.

[3]Test dataset corrected for mean bias of CO_2 production.

Model 2 ("on-farm model"): CO_2 (g/d) =

$$b_0 + (b_1 \times \text{ECM}) + (b_2 \times \text{BW}^{0.75}) + (b_3 \times \text{Milk CF}) + (b_4 \times \text{DIM}) + \text{breed} + (b_{\text{DIM,DietCF}} \times \text{DIM} \times \text{Diet CF}) + (b_{\text{ECM,DIM}} \times \text{ECM} \times \text{DIM}) + (b_{\text{ECM,BW}^{0.75}} \times \text{ECM} \times \text{BW}^{0.75}) + (b_{\text{MilkCF,BW}^{0.75}} \times \text{Milk CF} \times \text{BW}^{0.75}) + (b_{\text{BW}^{0.75},\text{breed}} \times \text{BW}^{0.75}) + (b_{\text{DIM,breed}} \times \text{DIM}) + (b_{\text{BW}^{0.75},\text{parity}} \times \text{BW}^{0.75}),$$

where, b_0 is the intercept; and b_1, b_2, b_3, and b_4 are the coefficients of ECM (kg/d), $BW^{0.75}$ ($kg^{0.75}$), milk CF (g/kg milk), and DIM (d), respectively. Furthermore, $b_{DIM,DietCF}$, $b_{ECM,DIM}$, $b_{ECM,BW^{0.75}}$, and $b_{MilkCF,BW^{0.75}}$ are the coefficients of DIM × diet CF, ECM × DIM, ECM × $BW^{0.75}$, and milk CF × $BW^{0.75}$, respectively, and $b_{BW^{0.75},breed}$ and $b_{DIM,breed}$ are the breed-specific coefficients of $BW^{0.75}$ and DIM, whereas $b_{BW^{0.75},parity}$ is the parity-specific coefficient of $BW^{0.75}$. All coefficients are listed in Table 4.

An example of using model 2 to calculate the CO_2 production from a second-parity Ayrshire cow, with a yield of 30 kg ECM/d, weighing 650 kg, being 110 DIM, with an average milk CF concentration at 35.0 g/kg milk, eating a TMR with a CF content at 40 g/kg DM is as follows:

$$-6{,}134 + [213 \times 30\ (\text{kg ECM/d})] + [126 \times 650\ (\text{kg}^{0.75})] + [52.5 \times 35.0\ (\text{g/kg})] + [-5.13 \times 110\ (\text{d})] + 0 + [-0.122 \times 110\ (\text{d}) \times 40\ (\text{g CF/kg DM})] + [0.386 \times 30\ (\text{kg ECM/d}) \times 110\ (\text{d})] + [-1.18 \times 30\ (\text{kg ECM/d}) \times 650\ (\text{kg}^{0.75})] + [-0.614 \times 35.0\ (\text{g milk CF/kg}) \times 650^{0.75}\ (\text{kg})] + [0 \times 650\ (\text{kg}^{0.75})] + [0 \times 110\ (\text{d})] + [3.66 \times 650\ (\text{kg}^{0.75})] = 11{,}634\ \text{g } CO_2 \text{ per day}.$$

Table 6. Model evaluation of the 3 models to predict CO_2 production (g/d) from lactating dairy cows based on the training dataset itself (n = 2,244), where model 1 is "best model," model 2 is "on-farm model," and model 3 is "reduced on-farm model"[1]

Item[2]	Test data et	RMSE	RMSE, % mean	MAE	CCC	RSR	MB	SB	MB, % MSE	SB, % MSE	Dispersion, % MSE
Model 1	Training dataset	1,435	11.6	1,115	0.73	0.71	−298	−0.18	4.32	5.27	90.4
Model 2	Training dataset	1,549	12.5	1,230	0.63	0.77	59.0	−0.14	0.145	1.87	98.0
Model 3	Training dataset	1,573	12.7	1,254	0.59	0.78	68.7	−0.071	0.191	0.382	99.4

[1]RMSE = root mean square error, MAE = mean absolute error, CCC = concordance correlation coefficient, RSR = ratio of RMSE to standard deviation of measured data, MB = mean bias, SB = slope bias, and MSE = mean square error.

[2]Model performance, training dataset based on observed CO_2 production.

Model 3 was intended for an on-farm setting, where BW is not a part of the predictor variables ("reduced on-farm model"). It reads,

$$\textbf{Model 3} \text{ ("reduced on-farm model"): } CO_2 \text{ (g/d)} = b_0 + (b_1 \times \text{ECM}) + (b_2 \times \text{DIM}) + \text{breed} + \text{parity} + (b_{\text{breed,parity}}) + (b_{\text{DIM,DietCF}} \times \text{DIM} \times \text{Diet CF}) + (b_{\text{ECM,DIM}} \times \text{ECM} \times \text{DIM}) + (b_{\text{DIM,breed}} \times \text{DIM}) + (b_{\text{MilkCF,parity}} \times \text{Milk CF}),$$

where b_0 is the intercept; b_1, b_2, and $b_{ECM,DIM}$ are the coefficients of ECM (kg/d), DIM (d), and ECM × DIM, respectively; $b_{DIM,DietCF}$ and $b_{ECM,DIM}$ are the coefficients of DIM × diet CF and ECM × DIM; $b_{DIM,breed}$ is the breed-specific coefficient of DIM; $b_{breed,parity}$ is the breed-specific coefficient for each parity; and $b_{MilkCF,parity}$ is the parity-specific coefficient of milk CF. All coefficients are listed in Table 4.

An example of using model 3 to calculate the CO_2 production from a first-parity crossbreed cow, with a yield of 28 kg ECM/d, being 100 DIM, eating a TMR with 35 g CF/kg DM, with 37 g CF/kg milk is as follows:

$$8{,}781 + [80.3 \times 28 \text{ (kg ECM/d)}] + [-4.66 \times 100 \text{ (d)}] - 1{,}237 + 0 + 0 + [-0.149 \times 100 \text{ (d)} \times 35 \text{ (g CF/kg DM)}] + [0.338 \times 28 \text{ (kg ECM/d)} \times 100 \text{ (d)}] + [11.3 \times 100 \text{ (d)}] + [-4.18 \times 37 \text{ (g milk CF/kg)}] = 10{,}727 \text{ g } CO_2 \text{ per day.}$$

The models predict the CO_2 production in grams per day; to calculate the CO_2 production in liters per day, see Materials and Methods section.

Evaluation on RC and GF Test Datasets

When evaluated on the RC test dataset, model 2 was superior with respect to RMSE, RMSE as percentage of mean, MAE, and RSR (Table 5). However, model 1 was superior with respect to MB, MB as percentage of MSE, and dispersion as percentage of MSE when evaluated on the RC test dataset.

Model 1 was superior in most of the evaluation parameters when the models were evaluated on the GF test dataset (RMSE, RMSE as percentage of mean, MAE, CCC, RSR, SB, and SB as percentage of MSE).

Model 3 performed better than models 1 and 2 with respect to SB, and SB as percentage of MSE, when evaluated on the RC test dataset. In addition, model 3 was superior to models 1 and 2 with respect to MB, MB as percentage of MSE, and consequently dispersion as percentage of MSE on the GF test dataset.

Evaluation on GF+ Test Dataset

Model 3 had the highest dispersion as percentage of MSE when the models were evaluated on the GF+ test dataset, as a consequence of low MB and MB as percentage of MSE (Table 5). Oppositely, RMSE, RMSE as percentage of mean, MAE, CCC, RSR, and SB were better for model 1 when evaluated on the GF+ test dataset.

Evaluation on the Modified Test Dataset

The predicted CO_2 production underestimated the actual measured CO_2 production in RC (MB across models was 1,315) and overestimated the actual measured CO_2 production using GF units (MB across models was 511). Bearing in mind that the models were developed with a training dataset containing both GF (40% of the records) and RC data (60% of the records), it was decided to address this by evaluating the 3 models with a modified dataset (see Materials and Methods section). The evaluations obtained with the modified test dataset clearly illustrated that nearly all the variation was related to dispersion error (Table 5).

Evaluation on the Training Dataset

Due to the risk of some of the models simply matching the properties of test dataset better than other models, it was decided to evaluate the models on the training dataset as well (Table 6). Furthermore, this evaluation illustrates the predictability of the models if certain animal parameters were not available. Model 1 was superior to the other models with respect to RMSE, RMSE as percentage of mean, MAE, CCC, and RSR, when evaluated on the training dataset. However, the actual values of MB or SB, and MB, SB, or dispersion as percentage of MSE for model 1 were not superior to model 2 and 3. This was partly caused by the relatively higher MB for model 1; SB was also slightly higher for model 1, causing the dispersion as percentage of MSE to be somewhat lower than it was for models 2 and 3. Model 2 (without DMI, with BW as predictor variable) performed slightly better than model 3 (without DMI and BW as predictor variables) with respect to RMSE, RMSE as percentage of mean, MAE, CCC, RSR, MB, and MB as percentage of MSE. However, SB and SB or dispersion as percentage of MSE were slightly better for model 3. Based on the comparison of model 2 and 3 on the training dataset, predicting CO_2 production from dairy cows in settings without data on BW is feasible.

DISCUSSION

Overall Model Evaluation on the Test Dataset

The models were developed with a training dataset where 69.3% of the records were Holstein cows, whereas Ayrshire, Jersey and others or crossbreed cows constituted 4.3%, 2.9%, and 23.5% of the records, respectively. The external validation test datasets consisted of only Holstein cows. Furthermore, the models were developed and evaluated with a dataset of cows having ≤300 DIM. It is important to consider this when applying the models to breeds other than Holstein cows or cows in lactation beyond 300 d.

Initially, the RC and the GF test datasets were treated as a unified dataset (data not shown), but a systematic underprediction for the RC data and a simultaneous overprediction for GF data were observed. Therefore, the models were evaluated separately on the RC and GF parts of the test dataset (Table 5). Additionally, the models were evaluated on the GF+ test dataset to investigate the potential impacts of the use of nitrate, 3-NOP, or a combination of nitrate and 3-NOP on the precision and accuracy of the models. The observed underprediction for RC and overprediction for GF could be caused by inherent model characteristics, but the method of gas measurement was confounded with research location (included as a random effect in all the models). Technical differences between the 2 methods, such as the GF units exclusively measuring gases emitted in exhaled air and not from the rectum of the cow, could partly contribute to the observed discrepancies, despite lack of data related to CO_2 released from the rectum of the cow. In addition, the GF relies on repeated short-term measurements, typically lasting 2 to 7 min, and repeated at intervals over subsequent days, whereas RC measurements are generally continuous over successive days (typically 2 to 4 d). The GF system has the advantage of being able to record gas data on a much larger number of animals compared with RC systems. Previous studies have compared RC and GF measurements of CO_2 emission, but the conclusions drawn were limited by the low number of animals (Doreau et al., 2018) and occasional reductions in DMI when cows entered the chambers (Alemu et al., 2017). For CH_4 production, Hristov et al. (2018) showed an unexpectedly weak relationship between DMI and CH_4 production measured with the GF (13.9 to 35.4 kg DMI, $R^2 = 0.05$), and a much stronger relationship measured with the RC (3.9 to 33.5 kg DMI, $R^2 = 0.58$), indicating a better capability of RC compared with GF to capture variation in gaseous release. However, the variation in DMI was also greater for the RC than the GF data, which could partly explain the better relationship for RC data in the study by Hristov et al. (2018); even a more restricted range of DMI (15.0 to 33.5 kg/d) with the RC still showed a stronger relationship ($R^2 = 0.41$) than with the GF.

The model performance was better when the 3 models were assessed based on the GF and the GF+ test datasets, compared with evaluation on the RC test dataset. This is evident from the higher dispersion in percentage of MSE and CCC (except for model 2 on GF+ test dataset). Moreover, the RMSE, RMSE as percentage of mean (except for model 2 on GF+ test dataset), and MAE were consistently lower when the models were tested on the GF and the GF+ test datasets. The major reason for lower model performance with the RC test data is the more pronounced MB (inaccuracy) compared with the GF test data. The mean of CO_2 production in the RC test dataset (14,369 g/d) is also higher than in the training dataset (12,402 g/d), likely contributing to the high MB observed when evaluating the models on the RC test dataset. The higher dispersion in percentage of MSE indicates that a greater fraction of variation is random variation for GF and GF+ (Table 5). However, it is important to acknowledge that these differences are also attributable to the sizes of the respective test datasets (Doreau et al., 2018), with the GF and GF+ test datasets being larger than the RC test dataset.

The DMI in the GF+ dataset (based on a part of 2 production trials) was on average lower than DMI in the GF dataset (based on the same 2 production trials, with all observations in it, plus 2 other production trials), likely causing the lower mean CO_2 production (g/d) in that specific test dataset (Table 3). Furthermore, the variation of the CO_2 production within the GF+ dataset was less (smaller SD: Table 3) than for the RC and GF test dataset, and the cows were earlier in lactation (mean DIM was lower for GF+ than RC and GF: Table 3). The evaluation on the GF+ test dataset should therefore be interpreted bearing in mind that the cows in this test dataset generally produced lower amounts of CO_2 with less variation; thus the evaluation on the GF+ test dataset indicates somewhat better model performance than the evaluation on the RC and GF test dataset.

All 3 models showed a low SB both in absolute values and as percentage of MSE when tested on the RC, GF, and GF+ test dataset (Table 5). This suggests consistently good prediction abilities for determining whether a given cow emits lower or higher amounts of CO_2 as compared with the average cow (Figure 1). Furthermore, it indicates that GF units rank cows with comparable precision to RC, contrasting with the results from a previous study (Alemu et al., 2017) but partially in agreement with the findings of Rischewski et al. (2017). Precision is of importance, especially when the models are used within a herd to rank individual cows based on their CO_2 production, and subsequently ranking them according to their CH_4 production by combining estimated CO_2 pro-

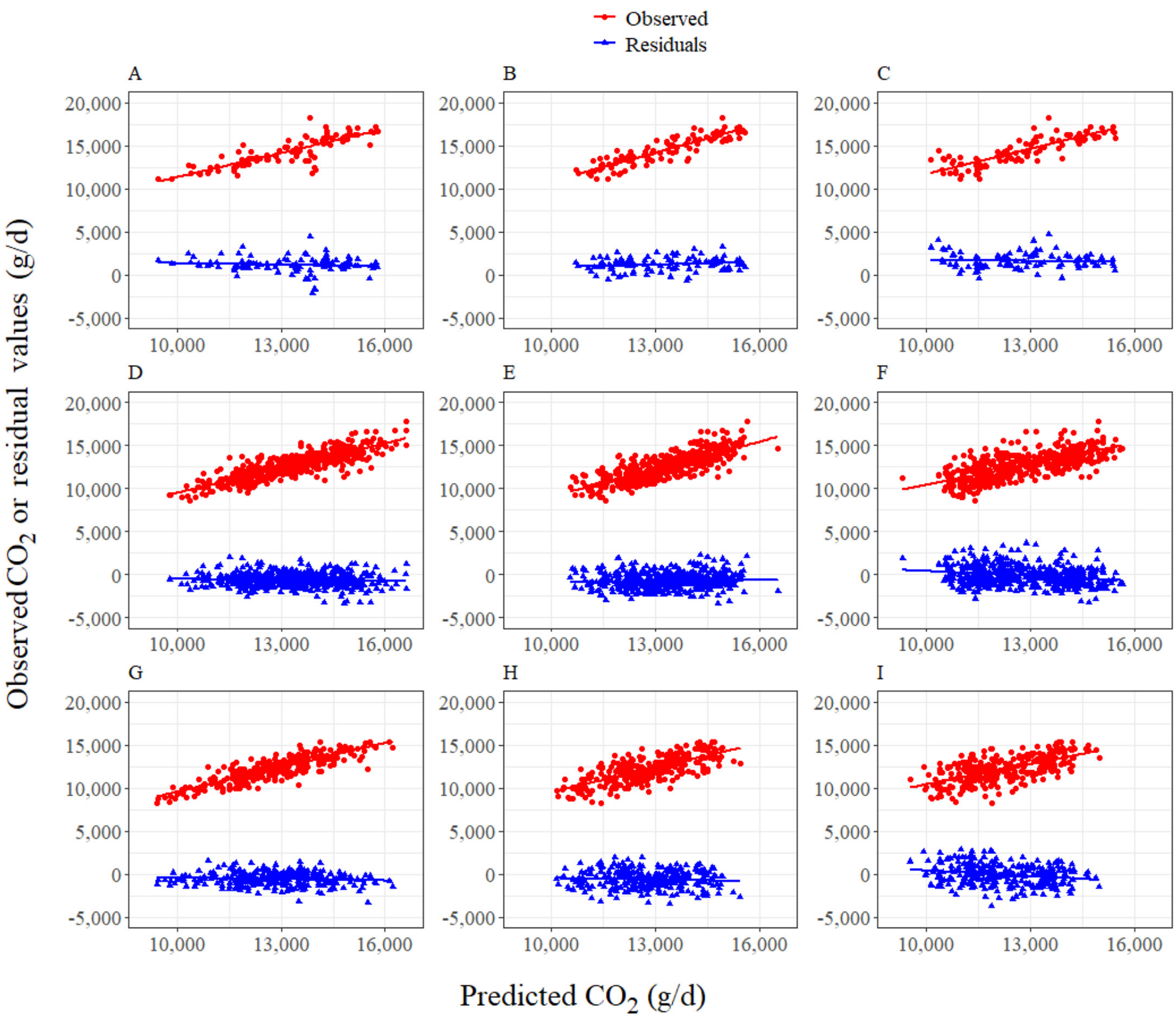

Figure 1. Observed (red) CO_2 production (g/d) in the test dataset, and residual values (blue) of CO_2 production of the 3 models plotted against predicted values of CO_2 production for (A) model 1 ("best model"), tested on respiration chamber (RC) data; (B) model 2 ("on-farm model"), tested on RC data; (C) model 3 ("reduced on-farm model"), tested on RC data; (D) model 1, tested on GreenFeed (GF) data; (E) model 2, tested on GF data; (F) model 3, tested on GF data; (G) model 1, tested on GF data, only including diets containing nitrate, 3-nitrooxypropanol (3-NOP), or both nitrate and 3-NOP (GF+); (H) model 2 tested on GF+ data; and (I) model 3 tested on GF+ data. The red and blue line represent the linear regression lines of observed and residual values, respectively.

duction and measured $[CH_4]$:$[CO_2]$ ratio in breath using the sniffer technique. However, model 1 and model 2 had noticeable MB when evaluated on the RC and GF dataset, indicating a lack of accuracy and a disparity in absolute values between RC and GF. Assuming that RC data represents the true production of CO_2, and that RC are seen as the the gold standard, it is suggested to add the MB from the RC evaluation of the given model to the dependent variable of the model (CO_2 g/d). This would cause the outcome of the models to reach a more accurate level, if the observed difference between RC and GF data in the current test dataset is considered universal across research groups.

A slight underprediction of CO_2 production for the measured low levels of CO_2 production was evident from the regression lines of the present models on a reduced version of the training dataset where DIP was given (n = 562, Figure 2). However, the underprediction was even

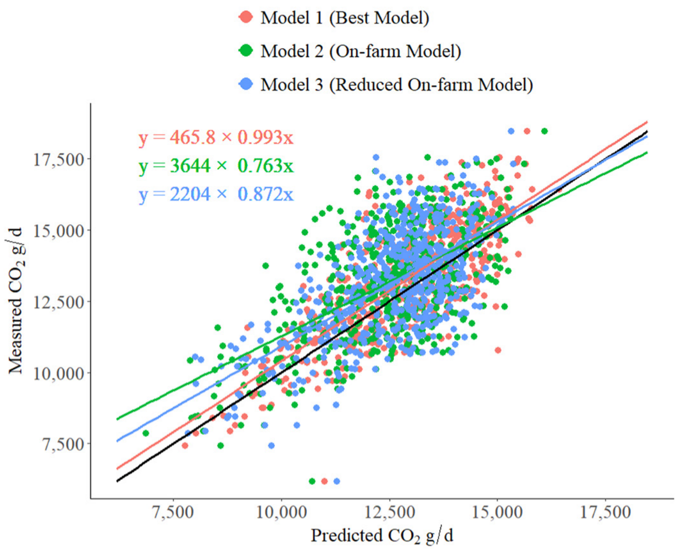

Figure 2. Measured CO_2 production (g/d) plotted against predicted CO_2 production in a reduced version of the training dataset (n = 562, see Supplemental Table S3), where observations having missing values of days in pregnancy were not included. The black line represents y (measured CO_2 production, g/d) = x (predicted CO_2 production, g/d); the red, green, and blue lines represent linear regressions of CO_2 production predicted by models 1, 2, or 3, respectively. This reduced version of the training dataset was a part of the training dataset (n = 2,244), which these 3 models were derived from. Regression lines are given for each model.

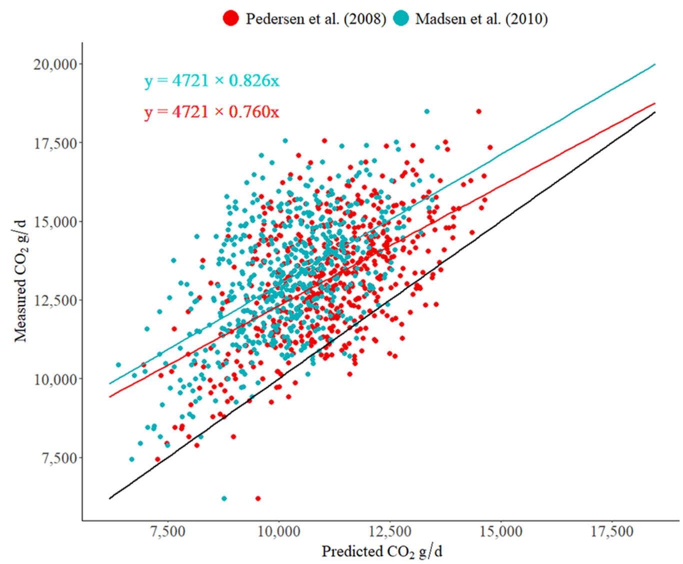

Figure 3. Measured CO_2 production (g/d) plotted against predicted CO_2 production in a reduced version of the training dataset (n = 562, see Supplemental Table S3), where observations having missing values of days in pregnancy were not included. The black line represents y (measured CO_2 production, g/d) = x (predicted CO_2 production, g/d); the red and blue lines represent linear regressions of CO_2 production predicted by Pedersen et al. (2008) and Madsen et al. (2010), respectively. Regression lines are given for each model.

more pronounced when using the previous equations from Madsen et al. (2010) and Pedersen et al. (2008) on the same reduced dataset (Figure 3). This advocates for using the models from the present study in combination with the sniffer method instead of the equations from Madsen et al. (2010) and Pedersen et al. (2008).

Gestation

Previously, the requirement of metabolizable energy (**ME**) for pregnancy in dairy cows was described by an exponential function related to number of days pregnant, with an efficiency of 10.5% of ME for fetal tissue deposition (Moe and Tyrrell, 1972). A recent study estimated efficiency of ME for pregnancy in Holstein × Gyr heifers to be 14.1% (Sguizzato et al., 2020). However, this estimation was based on a nonlinear development of net energy (**NE**) for pregnancy, and possible variation was not taken into account (Sguizzato et al., 2020). According to Nielsen and Volden (2011), the NE requirement for gestation is only minor when DIP <150, but significantly increased for cows >150 DIP. Thereby HP increases along gestation, assuming a constant efficiency of ME for gestation as indicated in Moe and Tyrrell (1972) and Sguizzato et al. (2020). The gravid uterus and development of the mammary gland cause HP to increase (Sguizzato et al., 2020), as they, especially the gravid uterus, account for significant metabolism of nutrients. Hence, DIP was initially included in the models (and it is a factor in the equation of HP; CIGR, 2002). However, only a few records in the training dataset (n = 562) could provide such data, likely due to lack of recording, and DIM was expected to be used as a close proxy for DIP. However, in the part of the training dataset with DIP available (n = 562 records), DIM was not a very precise indicator of DIP (Supplemental Table S2, see Notes), likely because the time point of a successful insemination of these experimental animals did not follow the same pattern across research locations. Therefore, the effect of DIP as a predictor variable was only tested on the smaller dataset where DIP was available, and there was no effect of DIP on CO_2 production ($P = 0.30$).

Dietary Crude Protein

Increasing the dietary CP level has been found to increase the energy content in cattle urine (Ramin and Huhtanen, 2013; Hynes et al., 2016), and the energy content in cattle urine is closely linked with the urinary carbon content (Morris et al., 2021). In addition, dietary CP is an indicator of nutrient composition within a diet. In the current training dataset, data on NDF and starch content were not collected from the research locations, and the correlation between dietary CP and CF was low

(−0.07). However, dietary CP was positively correlated with urinary nitrogen (g/d) in van Lingen et al. (2018), and in the same study, urinary nitrogen (expressed in g/kg DMI) was positively correlated with CH_4 yield. A part of the explanation could be a higher DM digestibility when cows are sufficiently supplied with dietary CP (Oldham, 1984). Thereby, more nutrients are available for intermediary metabolism without increasing the DMI. Excess of absorbed amino acids also causes alterations in oxidation, and less efficient conversion of ME to NE, thereby increasing CO_2 production (Oldham, 1984). Not surprisingly, dietary CP concentration was therefore a significant predictor variable of CO_2 production in model 1 ("best model"), where DMI was also included in the model, and increased dietary CP intake increased CO_2 production.

Effects of Different CH_4-Mitigating Additives or Feedstuffs on CO_2 Production

A recent study has shown decreased CO_2 production and increased CO_2 yield (g/kg DMI; Kjeldsen et al., 2024) when dairy cows were fed 3-NOP, even though 3-NOP, by its mode of action, is not expected to affect CO_2 metabolism, except from a small increase due to less reduction of CO_2 to CH_4. These results are in alignment with another study where increased CO_2 yield for 3-NOP concentrations of both 60 mg/kg DM (+3%) and 80 mg/kg DM (+4%) were observed, whereas it was only the diet with 80 mg/kg DM that negatively affected CO_2 production (van Gastelen et al., 2022). Additionally, Maigaard et al. (2024) observed a reduced CO_2 production and increased CO_2 yield when cows were provided 80 mg 3-NOP/kg DM. In the 3 studies mentioned above, DMI was negatively affected by 3-NOP supplementation for reasons still unclear, which likely at least partly caused the effect on CO_2 yield and production. Melgar et al. (2021) and Van Wesemael et al. (2019) did not observe decreased DMI when dairy cows were supplemented with 3-NOP, nor changes in CO_2 production or yield; this indicates that a reduction in CO_2 production associated with the use of a given potent CH_4-mitigating feed additive seems to be related to a potential reduction in DMI.

Nitrate acts as an alternative hydrogen sink and competes with methanogens in taking up H_2 in the rumen (Leng, 2008). Considering the mode of action, nitrate supplementation does not affect CO_2 metabolism of the animal, as also not found in the study by Olijhoek et al. (2016), where 5, 14, and 21 g nitrate/kg DM were fed to the cows. However, Wang et al. (2023) included 10 g nitrate/kg DM and observed decreased CO_2 production, when dairy cows were supplemented with nitrate, although likely due to reduced DMI.

Increased dietary fat content has also proven to be an effective CH_4 mitigation strategy (Beauchemin et al., 2007). The training dataset reflects very different feed rations, and thereby CF levels also varied, from 12 to 74 g/kg DM (Table 2). Metabolism of fat releases more heat (28 kJ/L CO_2) than the metabolism of carbohydrates (21 kJ/L CO_2; Madsen et al., 2010). However, increasing the fat level from 2% to 5% of the diet reduces CO_2 production by ~1 percentage unit (Madsen et al., 2010), as the efficiency of using ME to NE of lactation is relatively high (estimated to 0.63 in Moraes et al., 2015, and 0.60–0.64 in Moe, 1981), and thus less heat is lost with feeding higher fat concentrations, as long as the mammary gland takes up the fatty acids provided by the feed. The study by Maigaard et al. (2024) is one of few to report CO_2 emissions when feeding a high level of fat (60–67 g dietary CF/kg DM). They reported a significant effect of fat supplementation on CO_2 production, but an interaction was observed between fat and nitrate supplementation, and interpretation of the results are affected by this interaction. In conclusion, high (>60 g/kg DM) or low (<30 g/kg DM) CF concentrations of a given diet are not expected to cause less precise estimation of the CO_2 production in the current study.

CONCLUSIONS

Production of CO_2 (g/d) from lactating dairy cows can be predicted directly from dietary, animal, and production traits, without quantifying HP. The absolute values of SB (−0.22 to 0.097) and SB as percentage of MSE (0.049 to 4.89) were very low, which indicates precision of the models. The absolute value of the dependent variable (CO_2 g/d) should be interpreted accounting for the fact that the models were developed on a dataset containing both RC and GF data, causing a relatively high MB for nearly all models in all evaluations (−740 to 1,619).

NOTES

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Nonstandard abbreviations used: 3-NOP = 3-nitrooxypropanol; $BW^{0.75}$ = metabolic BW; CCC = concordance correlation coefficient; CF = crude fat; DIP = days in pregnancy; GF = GreenFeed head chamber; HP = heat production; HPU = heat-producing unit; MAE = mean absolute error; MB = mean bias; ME = metabolizable energy; MSE = mean square error; NE = net energy; RC = respiration chamber; RMSE = root mean square error; RSR = ratio of RMSE to SD of measured data; SB = slope bias.

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