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Original research article

A mathematical model to describe the diurnal pattern of enteric methane emissions from non-lactating dairy cows post-feeding

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ABSTRACT

Enteric methane emission is not only a source of energy loss in ruminants, but also a potent contributor to greenhouse gas production. To identify the nature and timing of interventions to reduce methane emissions requires knowledge of temporal kinetics of methane emissions during animal husbandry. Accordingly, a mathematical model was developed to investigate the pattern of enteric methane emissions after feeding in dairy cows. The model facilitated estimation of total enteric methane emissions (V, g) produced by the residual substrate (V_1, g) and newly ingested feed (V_2, g). The model was fitted to the 10 h methane emission patterns after morning feeding of 16 non-lactating dairy cows with various body weights (BW), and the obtained parameters were used to predict the kinetics of 24 h methane emission for each animal. The rate of methane emission (g/h) reached a maximum within 1 to 2 h after feeding, followed by a gradual post-prandial decline to a basal value before the next feeding. The model satisfactorily fitted curves for each cow according to the criterion of goodness-of-fit, and provided biological descriptions for fluctuations in methane emissions based on basal V_1 and feeding V_2 in response to the changes in BW and dry matter intake (DMI) of different dairy cows. The basal V_1 and feeding V_2 are probably maintained by slow- and readily-degradable substrates, respectively. The former contributed at least 0.6 of methane production. In summary, the model provides a means to separate basal V_1 and feeding V_2 within V, and can be used to predict 24 h emission from a single feeding period.

1. Introduction

The rumen is an important organ for degradation of feeds to produce volatile fatty acids (VFA), ammonia, and methane and for the production of microbial cells. Methane emissions represent an energy loss of 2 to 12% of gross energy intake (Johnson and Johnson, 1995). High enteric methane emissions not only indicate an inefficiency of energy utilization by the animal, but also are a potent source of greenhouse gases that trap heat in the atmosphere. Over recent decades, a number of mathematical models have been developed to estimate the amount of daily enteric methane production in ruminants, based on either mechanistic or regression equations (Bannink et al., 2011; Benchaar et al., 1998; Ellis et al., 2009). However, few studies have been conducted using models to investigate the diurnal pattern of enteric methane emissions from ruminants. Such information may be important as various strategies are considered to reduce methane production by ruminants.

Methane is produced through the activity and growth of methanogens in the rumen, and the diurnal pattern of enteric methane emissions is dependent on both the amount of feed ingested and the feeding pattern (IPCC, 2006; Johnson and Johnson,

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1995; Martin et al., 2010). Enteric methane emissions increase after feeding, reach a maximum and then gradually decrease to the pre-feeding (basal) level (van Zijderveld et al., 2010, 2011). The aim of the present study was to develop a model to describe the temporal pattern of enteric methane emissions and thus identify the contribution of the various processes to total methane emission. Empirical data from non-lactating dairy cows were individually fitted to a mathematical model, and the corresponding parameters were used to predict daily methane emission in non-lactating cows. In particular, the proportional responses in basal and feeding methane emissions to variations in dry matter intake (DMI) and body weight (BW) were explored.

2. Materials and methods

2.1. The model

The parameters for the model development are summarized in Table 1. Methane is emitted during the metabolism of methanogens that use hydrogen as an energy source, and this hydrogen is produced mainly during fermentation of degradable substrate by microorganisms in the rumen (Wang et al., 2013a). Methane emission rate (dV/dt, g/h) is assumed to be proportional to methanogen mass (M_r, g), activity of methanogens and degradable substrate (S_r, g) in the rumen, and is expressed as:

$$\frac{dV}{dt} = \alpha\beta_M M_r S_r, \tag{1}$$

where α is a proportionality constant [/(h·g)], β_M is the activity of methanogens linking the methane production and methanogen mass (g/g).

The substrate in the rumen was separated into two components: newly ingested and the residue, representing potential nutrient sources from the current and previous feeding, respectively. The total enteric methane produced associated with these feed fractions was a combination of that produced from use of residual (basal) substrate (V_1) and newly ingested (V_2) feed in the rumen. Many studies indicate that the methanogens grow only slowly, and the population does not increase much within a 12 h time window (Khelaifia et al., 2013; Sakai et al., 2009). Indeed, it was reported that the population of methanogens had the density of 4×10^8 to 8×10^8/g of rumen content and remained constant post-prandially (Leedle and Greening, 1988). Therefore, the current model assumes that changes in methane emissions are a response to substrate supply and activity of the methanogens, while methanogen mass (M_r) was assumed fixed for an individual animal on a particular ration. The rate of enteric methane emission, thus, can be expressed as follows:

$$\frac{dV}{dt} = \frac{dV_1}{dt} + \frac{dV_2}{dt}, \tag{2}$$

$$\frac{dV_1}{dt} = \alpha_1\beta_{M1} M_r S_{rr}, \tag{2a}$$

$$\frac{dV_2}{dt} = \alpha_2\beta_{M2} M_r S_{lr}, \tag{2b}$$

where α_1 and α_2 are proportionality constants [/(h·g)] for basal V_1 and feeding V_2, respectively; β_{M1} is the activity of methanogens to generate basal V_1; S_{rr} is the amount of degradable substrate in the residue of rumen before feeding (g); β_{M2} is the activity of methanogens to generate feeding V_2; S_{lr} is the amount of degradable substrate in the rumen from the newly ingested feed (g).

Both S_{rr} and S_{lr} are impacted by the ruminal passage rate (k_p) (Dijkstra et al., 1992), and will be zero as '$t \to +\infty$'. As a result, the rate of methane emission will theoretically be zero at '$t \to +\infty$' based on Eq. (1). However, in the livestock husbandry, both S_{rr} and S_{lr} will not be zero under normal feeding regimes. In practice, a proportion of new feed ingested will become a part of S_{rr} in the next feeding, and contribute to the portion lost by passage rate. So, we assumed that the replacement (by feeding) and outflow of S_{rr} were in approximate balance under any specific feeding regime, so that the S_{rr} could be given a fixed value.

The activity of methanogens is positively correlated to hydrogen produced (Janssen, 2010). The hydrogen itself is positively correlated to the amount of degradable substrate in the rumen (Leedle

Table 1
Explanation of the selected terms during the development of the model.

Term	Unit	Explanation
V	g	Volume of enteric methane emission
V_1	g	Volume of enteric methane emission generated by the residual substrate in the rumen
V_2	g	Volume of enteric methane emission generated by the newly ingested feed
dV/dt	g/h	Rate of enteric methane emission
dV_1/dt	g/h	Rate of enteric methane emission for basal V_1
dV_2/dt	g/h	Rate of enteric methane emission for feeding V_2
α	/(h·g)	Proportionality constant
α_1	/(h·g)	Proportionality constant for basal V_1
α_2	/(h·g)	Proportionality constant for feeding V_2
β_M	–	Activity of methanogens
β_{M1}	–	Activity of methanogens to generate basal V_1
β_{M2}	–	Activity of methanogens to generate feeding V_2
S_r	g	Degradable Substrate in the rumen
S_{rr}	g	Degradable substrate in the residue in the rumen before feeding
S_{lr}	g	Degradable substrate in the rumen from the newly ingested feed
S_l	g	Degradable substrate from newly ingested feed
S_{le}	g	Degradable substrate from newly ingested feed which outflow from rumen
M_r	g	Methanogens in the rumen
k_p	/h	Ruminal passage rates
S_T	g	Potential degradable substrate in the newly ingested feed
VF_2	g	Final asymptotic accumulated enteric methane emissions for feeding V_2
γ	g/h	Shape parameter
d	–	Shape parameter
a	g	Shape parameter

and Greening, 1988). As both the characteristic and amount of residual substrate were assumed to have fixed values (c) under a specific feeding regime, the activity of methanogens to use residual substrate will be constant. Therefore, Eq. (2a) can be re-expressed as:

$$\frac{dV_1}{dt} = \alpha_1 \beta_{M1} M_r S_{rr} = c. \tag{3}$$

A part of degradable substrate from newly ingested feed will outflow from the rumen, and S_{Ir} represents the fraction of degradable substrate after allowing for such losses, and is calculated by:

$$S_{Ir} = S_I - S_{Ie}, \tag{4}$$

where S_I is the amount of degradable substrate from newly ingested feed (g); S_{Ie} is the amount of degradable substrate from newly ingested feed that outflows from the rumen (g).

The ruminal passage rate (k_p) of degradable substrate is proportional to the quantity of degradable substrate in the rumen respectively (Dijkstra et al., 1992). The S_{Ir} can be attained by substituting "$S_{Ie} = S_{Ir}k_p t$" to Eq. (4), and expressed as:

$$S_{Ir} = S_I/(1 + k_p t). \tag{5}$$

The methane emission rate generated by the new feed ingested is attained by combing Eqs. (2b) and (5), and re-expressed as:

$$\frac{dV_2}{dt} = \frac{\alpha_2 \beta_{M2} M_r S_I}{1 + k_p t}. \tag{6}$$

The contribution of increased rate of enteric methane emission after a meal maybe due to more metabolically active methanogens, because more hydrogen is available (Robinson et al., 1981). So, the activity of methanogens to use H_2 from newly ingested feed is not constant, and positively correlates to the amount of new degradable substrate available. The initial starting value of β_{M2} should be equal to β_{M1} of methanogens in the residual substrate. A constant (Y_G, /g) was set to link the increased activity of methanogens and the amount of degraded substrate of the newly ingested feed and expressed as:

$$Y_G = \frac{\beta_{M2} - \beta_{M1}}{S_T - S_I}, \tag{7}$$

where S_T is the total amount of potential degradable substrate in the new feed provided.

Another constant (Y_S) was set to link the amount of degraded substrate of newly ingested feed and the volume of methane emission and expressed as:

$$Y_S = \frac{V_2 - VS_2}{S_T - S_I}, \tag{8}$$

where VS_2 is hypothetical starting enteric methane emissions (g) generated from the newly ingested feed.

The Eq. (6) can be solved by substituting Eqs. (7) and (8) into (6), and re-expressed as:

$$\begin{cases} \dfrac{dV_2}{dt} = \dfrac{\gamma\left(\dfrac{V_2}{VF_2} + d\right)\left(1 - \dfrac{V_2}{VF_2}\right)}{1 + k_p t} \\ VF_2 = S_T Y_S \\ d = \dfrac{Y_S \beta_{M1}}{Y_G VF_2} - \dfrac{VS_2}{VF_2} \\ \gamma = \dfrac{\alpha_2 Y_G M_r VF_2^2}{Y_S}, \end{cases} \tag{9}$$

where VF_2 is the final asymptotic accumulated enteric methane emissions (g) generated from the newly ingested feed, γ is a shape parameter (g/h), and d is a dimensionless shape parameter.

Eq. (9) can be re-expressed as:

$$\frac{dV_2}{\left(\frac{V_2}{VF_2} + d\right)\left(1 - \frac{V_2}{VF_2}\right)} = \frac{\gamma}{1 + k_p t} dt. \tag{10}$$

By integrating both sides of Eq. (10), the solution of Eq. (9) can be expressed as:

$$\begin{cases} V_2 = VF_2\left[\dfrac{1 + d}{z\exp(-a\ln(k_p t + 1)) + 1} - d\right] \\ z = \dfrac{1 - VS_2/VF_2}{VS_2/VF_2 + d} \\ a = \dfrac{\gamma(1 + d)}{k_p}, \end{cases} \tag{11}$$

where a is a shape parameter (g).

The methane emissions from degradable substrate in the residue of rumen before new feed is provided, and the methane emissions generated from newly ingested feed are set to be zero at '$t = 0$'. Then, Eq. (11) can be expressed as:

$$V_2 = VF_2\left[\frac{d + d^2}{\exp(-a\ln(k_p t + 1)) + d} - d\right]. \tag{12}$$

The first deviation of Eq. (12) represents methane emission generated by the newly ingested feed, and can be expressed as:

$$\frac{dV_2}{dt} = \frac{VF_2 a k_p (d^2 + d)(k_p + 1)[\exp(a\ln(k_p t + 1)) + d]}{[\exp(a\ln(k_p t + 1)) + d]^2 (k_p t + 1)}. \tag{13}$$

Methane emission rate can be attained by substituting Eq. (13) into Eq. (2), and expressed as:

$$\frac{dV}{dt} = \frac{VF_2 a k_p (d^2 + d)(k_p + 1)[\exp(a\ln(k_p t + 1)) + d]}{[\exp(a\ln(k_p t + 1)) + d]^2 (k_p t + 1)} + c. \tag{14}$$

The basal V_1 and total V can be attained by integrating Eqs. (2a) and (10), and expressed as:

$$V_1 = ct, \tag{15}$$

$$V = VF_2\left[\frac{d + d^2}{\exp(-a\ln(k_p t + 1)) + d} - d\right] + ct. \tag{16}$$

2.2. Animal and housing

The use of the animals and the experimental procedure were approved by the Animal Care Committee, Institute of Subtropical Agriculture. The experiment was conducted at a local farm in the Wang-Cheng County of Hunan Province, China. Sixteen non-lactating Chinese Holstein dairy cows with a wide range of BW (Table 2) were assigned to the air-flow controlled chamber for enteric methane emission measurement.

Cows were housed in a tie-stall dairy barn, and were accustomed to restricted movement. Both gaseous exchange and feed intake were individually determined when the cow was placed in the respiration chamber. Cows were allocated to the single respiration chamber for two consecutive days in a staggered manner. The data presented are averaged from the two days of chamber. The experiment lasted from early Feb. 2012 to late Apr. 2013.

2.3. Diet and feeding

The diet consisted of concentrate and roughage (rice straw). The concentrate contained maize, soybean meal, cottonseed meal and corn distiller's dried grains and maize with solubles, purchased from Agribrands Purina Feed mill Co., Ltd. The chemical composition of the concentrate was 950 g DM/kg and 155 g of CP, 415 g of neutral detergent fibre (NDF) and 157 g of acid detergent fibre (ADF) per kg of DM. The chemical composition for the rice straw was (on a DM basis) 975 g/kg DM, 63 g/kg CP, 760 g/kg NDF and 466 g/kg ADF.

The allowances of concentrate and roughage were decided by the farmer, based on experience and according to the live weight of individual cows (each around 1% of live weight). As a result, the amount of concentrate supplied was different for each animal (Table 2). The concentrate and roughage were placed in two separate feeding troughs, with the concentrate provided first. All animals had ad libitum access to water. The restricted supply of concentrate was divided into two portions for the morning and afternoon feeds (0600 and 1605 h) while the rice straw was provided in slight excess for both periods. Orts were collected twice daily before the new feed was provided. The characteristics of feed intake for all animals are shown in Table 2.

2.4. Measurement of methane emissions

One simple respiration chamber was built for the measurement of methane emissions from cows. Briefly, the chamber was made of galvanized steel plate with internal dimensions of 3 m length × 2 m width × 2 m height. The chamber had one front and one rear door fitted with internal rubber seals. The cow was restrained in the chamber with access to a feed bin and a drinking water container. A fresh air inlet was located at the top left of the chamber, and air inlets were piped from an intake vent, located 15 m from the chamber. The outlet consisted of two round polyethylene pipes (outside diameter, 50 mm) fixed to the left and right insides of the chamber, and each pipe comprised of 50 intake holes equally distributed around the entire circumference of the duct. These two ducts were piped through the right side of chamber via a 50 mm outside diameter polyethylene pipe. The outlet was connected via a 50 mm air filter, to a gas flow meter, followed by the pump. Airflow (150 to 190 m^3/h) under negative pressure was controlled by the pump. The chamber was fitted with four internal ventilation fans for efficient mixing of exhaled gases and incoming air. The outlet pipe from the chamber was connected to a plastic buffer box (50 cm length × 50 cm width × 50 cm height) for gas sampling.

The outlet gas was sampled from the box every 15 min during 0600 to 2200 h, at 2300, 2400 h, next day 0200 and 0530 h. A 50-mL syringe was used for sampling, and then injected into a vacuum tube for methane determination by gas chromatography (Agilent 7890A, Agilent Inc., Palo Alto, CA).

The cows were placed in the chamber at 0600 h. The cows were fed after entering the chamber at 0600 h, and the chamber was opened once a day at 1605 h for 5 min to deliver diet. The first sample of outlet gas was collected after the cows had been shut in the chamber for 10 min. Three inlet gas samples were collect at 0600, 1200 and 1700 h, and their mean value used to represent the methane concentration of the inflowing air.

Methane emission rate (R, g/h) and the total amount of methane emission (V, g) were calculated as follows:

$$\Delta V_i = 1000\left\{\frac{16.04R_g[(CO_i + CO_{i-1})/2 - CI](t_i - t_{i-1})}{22.4} + \frac{16.04V_c(CO_i - CO_{i-1})}{22.4}\right\}, \quad (17)$$

$$R_i = \frac{\Delta V_i}{t_i - t_{i-1}} = 1000\left\{\frac{16.04R_g[(CO_i + CO_{i-1})/2 - CI]}{22.4} + \frac{16.04V_c(CO_i - CO_{i-1})}{22.4(t_i - t_{i-1})}\right\}, \quad (18)$$

Table 2
Summary of variables for non-lactating dairy cows (n = 16).

Item	Mean	Median	Minimum	Maximum	SD
BW, kg	222	215	98	420	110
DMI, kg/d	4.45	4.44	2.66	7.35	1.38
DMI_a :DMI_m ratio	0.957	0.987	0.854	1.040	0.085
Concentrate, kg/d	2.96	3.22	1.61	4.02	0.82
Rice straw, kg/d	1.48	1.26	0.83	3.39	0.703
Concentrate proportion in the diet, %	66.8	68.2	53.9	78.8	7.55
NDFI, kg/d	2.30	2.20	1.37	4.14	0.781
ADFI, kg/d	1.18	1.09	0.70	2.23	0.426
CPI, kg/d	0.553	0.571	0.319	0.827	0.159
GEI, MJ/d	72.3	72.3	43.2	119	22.3
Methane, g/d	88.3	82.2	42.6	170	38.0
Methane, % of GEI	6.59	6.44	5.11	8.04	1.00

BW = body weight; DMI = dry matter intake; DMI_m = DMI for morning feeding from 0600 to 1600 h; DMI_a = DMI for afternoon feeding from 1600 to 0600 h; NDFI = neutral detergent fibre intake; ADFI = acid detergent fiber intake; CPI = crude protein intake; GEI = gross energy intake; SD = standard deviation.

$$V = \sum_{i=1}^{i=n} \Delta V_i, \tag{19}$$

where $\triangle V_i$ (g) is the amount of methane emission between t_{i-1} and t_i; R_g (m^3/h) is the flow rate of gas in the duct; R_i (g/h) is the rate of methane emission between t_{i-1} and t_i; CO_i (10^{-6} vol/vol) is the concentration of methane in the outlet air at time t_i and CO_0 is equal to CI; CI (10^{-6} vol/vol) is the concentration of methane in the inlet air; V_c (m^3) is the volume of chamber and is equal to 12 m^3; i is the number of sampling; n is the total number of samplings.

2.5. Model, parameters and statistics

As methanogens mainly use dissolved hydrogen in the liquid phase to produce methane (Robinson et al., 1981), the kp of degradable substrate in the rumen for methanogens can equal that of liquid. The kp employed was estimated using the empirical equation of kp liquid proposed by Seo et al. (2006), expressed as:

$$kp = 4.524 + 0.223FpBW + 0.2046CpBW + 0.344FDMI, \tag{20}$$

where $FpBW$ was the forage DMI as a proportion of body weight (g/kg); $CpBW$ was the concentrate DMI as a proportion of body weight (g/kg); $FDMI$ was the forage DMI (kg). The other three parameters, including VF_2, a, d and c were estimated by fitting the kinetics of methane emissions after morning feeding (0600 to 1600 h) with the model using NLREG version 5.4 software (Sherrod, 1995), and re-assigned to be VF_{2m}, a_m, d_m and c_m, respectively.

The feed intake after morning (0600 to 1600 h) and afternoon (1600 to 0600 h next day) feeding were slightly different (Table 2), and exhibited slightly different kinetics of methane emission (Figs. 1 and 2). The VF_2, a and d were re-estimated to predicted the kinetics of feeding V_2 after afternoon feeding from 1600 to 0600 h next day, and re-assigned to be VF_{2a}, a_a and d_a, respectively. The Y_G, Y_S, α_2, β_{M1}, M_r and kp were assumed to be same for kinetics of methane emissions after morning and afternoon feeding, due to the small differences in the amount of feed intake during the two periods of the day. The VF_{2a}, a_a and d_a for feeding V_2 from 1600 to 0600 h next day were estimated by combining Eqs. (9) and (11), and expressed as:

$$\begin{cases} VF_{2a} = VF_{2m}R \\ a_a = a_m R^2 \\ d_a = d_m/R, \end{cases} \tag{21}$$

where R was the ratio of DMI following the afternoon feeding (14 h) to that after the morning feeding (10 h).

The basal V_1, feeding V_2 and total V were calculated as:

$$\begin{cases} V_1 = 24c \\ V_2 = V_{2m} + V_{2a} \\ V = V_1 + V_2. \end{cases} \tag{22}$$

Mean square prediction error ($MSPE$), an indicator of overall deviation between the observed and predicted values, was calculated as:

$$MSPE = \sum_{i=1}^{n} (VP_i - VO_i)^2 \Big/ n, \tag{23}$$

where VP_i and VO_i are predicted and observed methane emissions at data point i respectively, and n is the number of data points defining each individual curve.

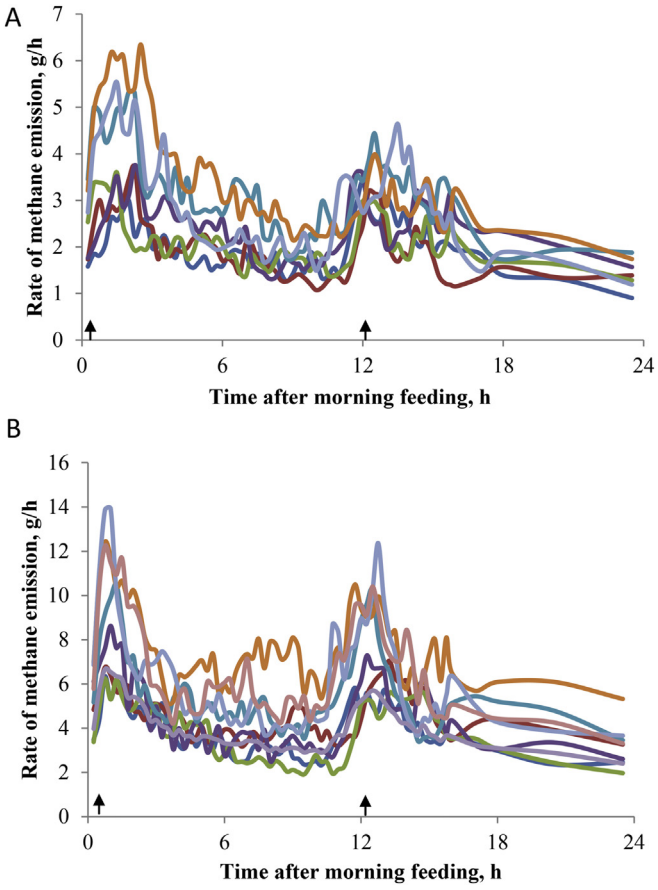

Fig. 1. Methane emission pattern (g/h) from sixteen non-lactating dairy cows for 24 h. Arrow was the time when the feed was provided. A and B were eight non-lactating dairy cows with body weight from 0 to 200 kg and 200 to 400 kg, respectively.

The root of mean square prediction error (r$MSPE$) is expressed as a percentage of the observed mean value (VO), and calculated as:

$$rMSPE = \sqrt{MSPE} \Big/ VO. \tag{24}$$

The MSPE is divided into three components resulting from bias, slope and random variation around the regression line (Bibby and Toutenburg, 1977; Dhanoa et al., 1999), which are calculated as:

$$\begin{cases} \text{bias} = (VP - VO)^2 \\ \text{slope} = (S_{VP} - rS_{VO})^2 \\ \text{random} = (1 - r^2)S_{VO}^2, \end{cases} \tag{25}$$

where VP and VO are the average predicted and observed methane emissions, respectively; S_{VP} and S_{VO} are standard deviations of predicted and observed methane emissions, respectively; r is correlation coefficient estimate, and calculated as:

$$r = \frac{1}{n} \sum_{i=1}^{n} (VO_i - VO)(VP_i - VP)/S_{VO}S_{VP}. \tag{26}$$

Concordance correlation coefficient analysis (CCC) was performed according to Lin (1989), where CCC is calculated as:

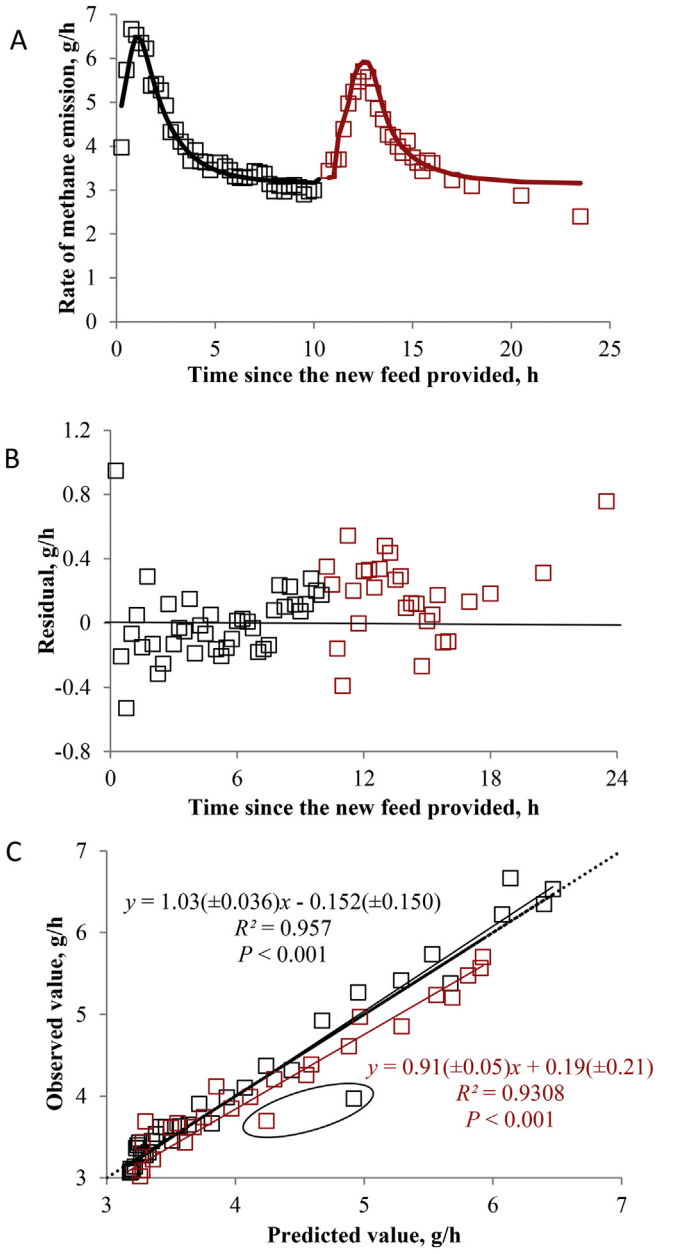

Fig. 2. Predicted versus observed rate of enteric methane emission. The observed and predicted rates of enteric methane emission are the average of 16 curves. A dotted line is unity of 1:1. Two points in the ellipse show disparity from the regression line. The data after morning and afternoon feeding were colored with black and red respectively. A and B were the diurnal pattern of rate of methane emissions and residual, respectively; C was predicted versus observed rate of enteric methane emission.

$$\begin{cases} CCC = rC_b \\ C_b = \dfrac{2}{S_{VO}/S_{VP} + S_{VP}/S_{VO} + \mu^2} \\ \mu = \dfrac{VO - VP}{(S_{VO}S_{VP})^{1/2}}, \end{cases} \quad (27)$$

where C_b is a bias correction factor, indicating the measure of accuracy; μ is the location shift relative to the scale, indicating the change in standard deviation. A positive μ-statistic indicates under prediction, while a negative μ-statistic indicates over prediction.

Linear regression between the predicted versus observed values was performed using ordinary least squares with SPSS 12.0 software (Chicago, IL, USA). As a result, statistical significance and regression equations were obtained by regression reports of SPSS 12.0 software (Wang et al., 2013b). The slope of residual linear regression (predicted values versus predicted−observed values) was tested for significance against zero using the method proposed by St-Pierre (2003) and performed in SPSS 12.0 software.

3. Results and discussion

3.1. Methane emission patterns after feeding

Fig. 1 describes the 24 h methane emission patterns of the non-lactating dairy cows after morning and afternoon feeding. Methane emission rate (V) varied widely between cows (range 1 to 14 g/h). There was an increase to the maximum 1 to 2 h after morning and afternoon feeding, followed by a gradual post-prandial decline. This temporal pattern of methane emissions agrees with other reports (van Zijderveld et al., 2010; van Zijderveld et al., 2011). In our study, similar values of methane emission ($P = 0.12$ using paired t-test) were observed at 10 h after morning and afternoon feeding (Figs. 1 and 2). Methane emissions were closely linked with DMI. Most of the feed provided (>60%) was ingested 1 to 2 h after feeding, and the concentrate provided was completely ingested within 1 h. Tolkamp et al. (2000) also reported that >60% of feed was ingested within 2 to 3 h after feeding.

3.2. The estimated parameters in the model

The estimated parameters in the model are shown in Table 3. The mean VF_{2m} was 8.95 g (range 2.36 to 18.2 g) and strongly affected by the DMI_m [Eq. (21)]. However, other factors, such as concentrate content in DMI_m and feeding behavior, might also affect VF_{2m} by altering the constant Y_G in Eq. (8). Other parameters such as a_m and d_m also varied widely (range 8.03 to 48.9 g and 0.026 to 0.295, respectively), because they are affected by a large number of factors according to the Eqs. (7) to (9) and (11). Further studies are needed to quantify Y_G, Y_S, α_2, β_{M1} and M_r for different feeds and animals to help in predicting VF_2, a and d [Eq. (14)]. As Y_G, Y_S, α_2, β_{M1} and M_r were set the same for both morning and afternoon feeding in each individual animal, DMI was the only factor to affect predicted VF_2, a and d based on the Eq. (14), and the results of prediction are shown in Table 3. The values of VF_{2a}, a_a and d_a were different to that of VF_{2m}, a_m and d_m, respectively, due to the slightly different DMI during the period of morning and afternoon feeding [Eq. (21)].

Table 3
Estimated parameters in the model.

Item	Mean	Median	Minimum	Maximum	SD
k_p, /h	0.0826	0.0817	0.0735	0.1013	0.0072
Parameters for kinetics of methane emission after morning feeding from 0600 to 1600 h					
VF_{2m}, g	8.59	8.90	2.36	18.2	3.92
a_m, g	21.7	19.2	8.03	48.9	11.3
d_m	0.121	0.114	0.026	0.295	0.073
Parameters for kinetics of methane emission after afternoon feeding from 1600 to 0600 h next day					
VF_{2a}, g	7.90	8.30	2.38	18.37	4.11
a_a, g	20.5	18.6	3.59	48.0	11.8
d_a	0.144	0.122	0.025	0.428	0.106

k_p = ruminal passage rate; VF_{2m} = the final asymptotic accumulated enteric methane emissions generated from the newly ingested feed after morning feeding; VF_{2a} = the final asymptotic accumulated enteric methane emissions generated from the newly ingested feed after afternoon feeding; a_m = shape parameter; d_m = shape parameter; a_a = shape parameter; d_a = shape parameters; SD = standard deviation.

3.3. Goodness-of-fit

The 10 h methane emission pattern after morning feeding was generally well-fitted by the model. The mean r was 0.908, with a range from 0.843 to 0.978 (Table 4). The mean MSPE was 0.283 with a range from 0.039 to 1.039, while the rMSPE was 12.1% with a range from 5.70 to 17.0% (Table 4). Most of the errors (> 97%) were due to unexplained random variance, based on MSPE (Table 4).

Table 4
Summary of the goodness-of-fit after fitting the curves of enteric methane emissions from dairy cows post-feeding (0 to 10 h).

Item	Mean	Median	Minimum	Maximum	SD
MSPE	0.283	0.227	0.039	1.04	0.283
rMSPE, %	12.1	12.5	5.70	17.0	2.80
Components of MSPE, %					
Slope	0.3	0.0	0.0	2.9	0.74
Random	99.7	100.	97.0	100	0.74
Bias	0.0	0.0	0.0	0.0	0.00
CCC	0.902	0.900	0.831	0.977	0.046
r	0.908	0.904	0.843	0.978	0.041
C_b	0.993	0.995	0.980	0.999	0.006
μ	0.000	0.000	0.000	0.001	0.000

MSPE = mean squared prediction error; rMSPE = root mean squared prediction error and expressed as a percentage of the observed mean value; SD = standard deviation; *CCC* = concordance correlation coefficient; r = correlation coefficient estimate; C_b = bias correlation factor; μ = location shift relative to the scale.

3.4. The predicted versus observed methane emissions

The accuracy of predicted methane emissions after afternoon feeding (10 to 20 h) was evaluated using the parameters derived from the 10 h methane emissions after morning feeding. In general, the accuracy of prediction decreased for the period of afternoon feeding, compared with the morning feeding (Tables 4 and 5). The mean r was 0.743 (0.515 to 0.938; Table 5). The mean MSPE was 0.254 (range 0.029 to 1.175), while the rMSPE was 11.5% (range 4.16 to 19.7%; Table 5). Most of the error was due to unexplained random variance (mean value = 70.4%) based on analysis of MSPE, although the maximum contributions of slope and bias to MSPE for some curves were 50.9 and 38.9%, respectively (Table 5). The negative μ-value (mean value = −0.199) indicated a slight over-prediction of methane emissions after afternoon feeding. Such μ-statistic is in agreement with the result of regression of observed versus predicted methane emissions in Fig. 3. In practice, the predicted fit of 10 to 20 h gave an over-estimate of 3.5% compared with observed but this increased to 10.1%, when 10 to 24 h was analyzed.

Table 5
The predicted versus observed curves of enteric methane emission from 10 to 20 h and 0 to 24 h.

Item	Mean	Median	Minimum	Maximum	SD
Methane emission from 10 to 20 h					
MSPE	0.254	0.127	0.029	1.175	0.290
rMSPE, %	11.5	11.1	4.16	19.7	4.63
Components of MSPE, %					
Slope	15.6	17.2	0.000	38.9	11.8
Random	70.4	74.8	43.5	97.5	15.9
Bias	13.8	8.12	0.066	50.9	15.8
CCC	0.689	0.704	0.338	0.919	0.189
r	0.743	0.770	0.515	0.938	0.134
C_b	0.919	0.960	0.696	0.999	0.097
μ	−0.199	−0.131	−0.853	0.339	0.319
Methane emission from 0 to 24 h					
MSPE	0.433	0.259	0.068	1.81	0.455
rMSPE, %	12.0	12.0	4.80	19.9	4.63
Components of MSPE, %					
Slope	4.39	4.58	0.021	14.0	3.70
Random	91.5	91.8	79.5	99.8	5.27
Bias	4.07	2.79	0.081	15.0	4.50
CCC	0.844	0.840	0.721	0.966	0.079
r	0.858	0.845	0.744	0.969	0.070
C_b	0.983	0.991	0.927	1.000	0.022
μ	−0.069	−0.053	−0.298	0.121	0.107

MSPE = mean squared prediction error; rMSPE = root mean squared prediction error and expressed as a percentage of the observed mean value; SD = standard deviation; *CCC* = concordance correlation coefficient; r = correlation coefficient estimate; C_b = bias correlation factor; μ = location shift relative to the scale.

The accuracy of predicted 0 to 24 h methane emissions is acceptable, in comparison to the observed methane emissions. The mean r was 0.858 (0.744 to 0.969; Table 5). The mean MSPE was 0.433 (range 0.068 to 0.1.81), while the rMSPE was 12% (range 4.8 to 19.9%; Table 4). Most of the error was due to unexplained random variance (> 79.5%) based on analysis of MSPE, although the maximum contributions of slope and bias to MSPE for some curves were 14.0 and 15.0%, respectively (Table 4). The negative μ-value (mean value = −0.069) also indicated a slightly over-prediction of daily methane from 0 to 24 h.

The predicted and observed methane emissions were separately pooled to give a mean of methane emissions from the 16 non-lactating dairy cows to allow examination of the residuals. Fig. 2A compared the predicted curves against the observed experimental values and the resulting residuals were plotted in Fig. 2B, which clearly indicates that the residuals were randomly distributed around zero line from 0 to 10 h and slightly lower than zero from 11 to 24 h. Such results were further confirmed by the zero and negative value of the μ-statistic in Tables 4 and 5, respectively. The regression slopes of the plot of predicted versus observed methane emissions were close to unity with values being 1.03 and 0.91 for morning and afternoon feeding, respectively, although two points showed obvious disparity (Fig. 2C). Furthermore, the slope of the plot of predicted versus 'predicted−observed' values showed a no significant slope against zero for periods of morning ($P = 0.33$) and afternoon ($P = 0.09$) feeding. The intercept of the plot was also not significantly different from zero for periods of morning ($P = 0.37$) and afternoon ($P = 0.37$) feeding.

The amount of daily methane emissions was further analyzed to investigate the accuracy of prediction using the parameters derived from only the 10 h methane emissions after morning feeding. Fig. 3 indicates that the accuracy of prediction is also high ($R^2 > 0.982$). The regression slope of the plot of predicted versus observed daily methane emissions was close to unity. The slope of the plot of predicted versus 'predicted−observed' values showed a no significant value ($P = 0.13$) against zero. Similarly, the intercept of the plot of predicted versus observed daily methane emissions was not significantly different ($P = 0.23$) to zero. Therefore, it appears that daily

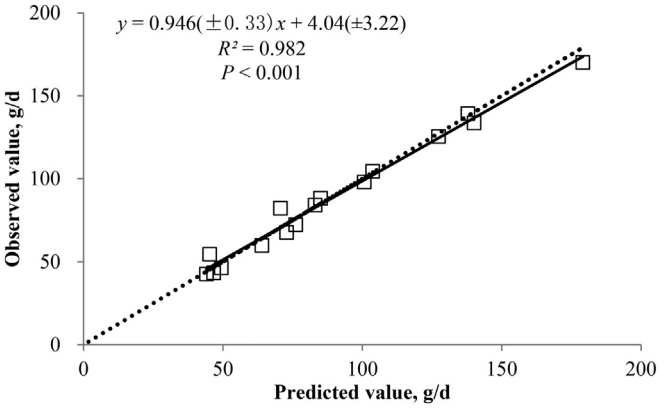

Fig. 3. Predicted versus observed daily enteric methane emission ($n = 16$). A dotted line is unity of 1:1.

methane emissions can be predicted with good accuracy by using just the 10 h methane emission pattern after morning feeding.

Relative large residuals occurred at the early stage (<1 h) after new feed was provided (Fig. 2A and B). These indicate that the fitted values were greater than the observed values at this early time. An assumption of the model is that hydrogen from the total amount of degradable substrate from feed intake is available for methanogens in the rumen immediately after new feed is provided. Nonetheless, it needs some time for animals to ingest feed (in practice only 60% of total feed is ingested by 1 to 2 h after feeding) and to mix within the rumen. So the real amount of the newly ingested feed in the rumen will be less than that assumed for the model at the early stages, leading to the smaller observed values in comparison to those predicted. Therefore, model accuracy can be further improved by incorporation of pattern of feed intake but this will involve further investigation.

Relative large residuals also occurred at the later stages of each feed interval. The basal methane emission was assumed to be a fixed value, based on the assumption that replacement and outflow of residual substrate were in approximate balance throughout. With this assumption then the rate of methane emission at 10 h after morning feeding should be equal that 10 h after afternoon feeding, at least as predicted by the model (Fig. 2A). If replacement is not equal to the outflow at the later stages after morning and afternoon feeding, then the predicted values will exceed observed, as shown by the direction of residuals and Fig. 2C. In the current study, the feeding times in morning and afternoon were10 and 14 h, respectively and the rate of methane emission at 14 h after afternoon feeding is lower than at 10 h after morning feeding, based on both the solution to Eq. (2) and empirical data in Fig. 2A. Using the kinetics of 10 h methane emission after morning feeding to predict kinetics of 14 h methane emissions after afternoon feeding caused a fixed bias (Fig. 2A and 2B). However, the bias did not reach significance concerning the prediction of methane emission rate and total daily methane emissions.

3.5. Biological interpretation of basal V_1 and feeding V_2

The enteric methane emissions were mathematically divided into two components (*i.e.*, basal V_1 and feeding V_2), as the basis for the model proposed. The V_1 and V_2 arise respectively from the residual substrate and newly ingested feed in the rumen. The basal V_1 are probably maintained mainly by the slow-degradable substrate, and also negatively correlated to the length of period between the current and next feeding. In contrast, the feeding V_2 mainly responds to the readily-degradable substrate, as can be observed after feeding high-concentrate diets (Leedle and Greening, 1988).

The slow intake for the roughage and rapid consumption of the concentrate fitted with the observed patterns in methane emissions. As the slow- and readily-degradable substrates mainly came from the roughage and concentrate, respectively, practical mitigation of methane emissions could be achieved by changing the ratio of basal V_1 to feeding V_2 through the use of more readily-degradable substrate intake. For example, supplementation of a diet with more readily-fermentable carbohydrates resulted in a decrease in methane emissions per unit of feed degraded (Moss et al., 2001; Ramin and Huhtanen, 2013), as did an increased proportion of concentrate in the diet (Aguerre et al., 2011; Boadi et al., 2004). On the other hand, decreasing feeding V_2 could be attained by inhibiting the activity of methanogens. Furthermore, dietary addition of nitrate and sulfate can deplete the availability of hydrogen for the growth of methanogens after feeding (van Zijderveld et al., 2010; van Zijderveld et al., 2011), leading to a reduction in feeding V_2 and total V.

The ratio of basal V_1 to total V exceeded 0.60 across 16 curves. This indicated that the large amount of methane was produced by the slow degradation of the roughage. In this context, reducing the basal V_1 might be preferred to altering feeding V_2 under farm conditions. Whether this could be easily achieved is not certain. For example, improved forage quality has led to lower (Chaves et al., 2006), higher (Ellis et al., 2012) or unchanged (Staerfl et al., 2012) methane emissions. Such differences might be related to the balance between additional precursor hydrogen production from water-soluble carbohydrates and decreased pH due to more VFA production (Staerfl et al., 2012). In addition, alteration of concentrate quality can decrease methane emissions by approximately 20% according to Hindrichsen et al. (2005).

On average, the concentrate content was 0.668 of total feed intake for all cows. Despite this, feeding V_2 was considerably less than basal V_1 (Figs. 4 and 5). It is known that influx of a great amount of readily-degradable substrate increases hydrogen

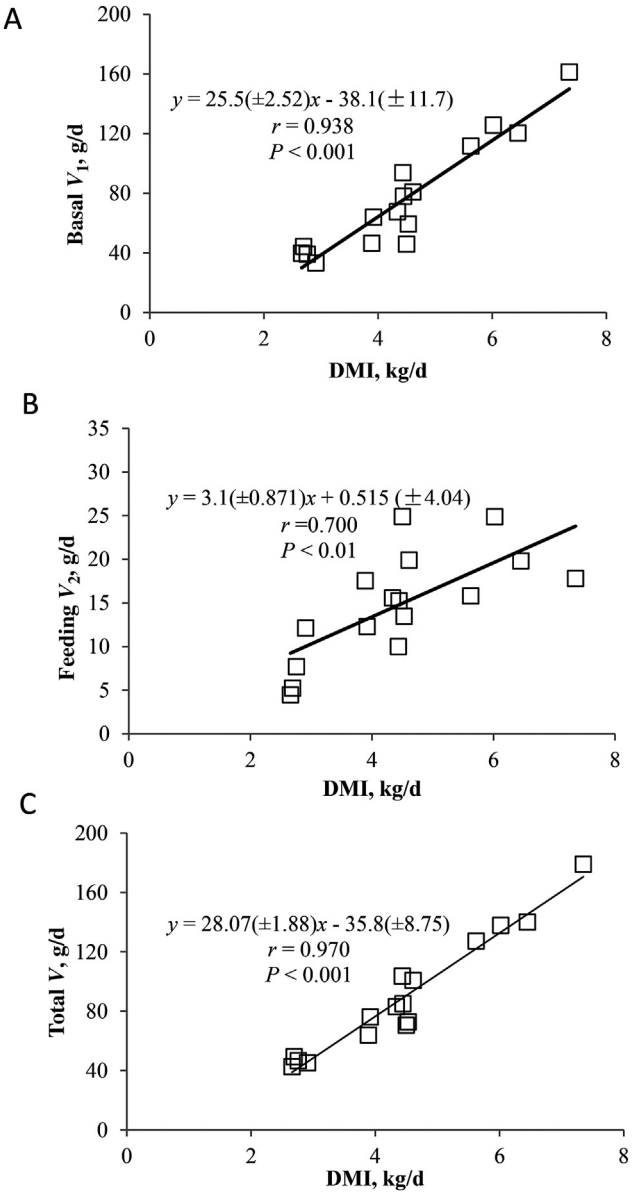

Fig. 4. Relationships between dry matter intake (DMI) and basal V_1 (A), feeding V_2 (B) and total V (C). The solid line shows the best-fit regression. V_1 = methane produced by residual substrate; V_2 = methane produced by newly ingested feed; V = methane emissions.

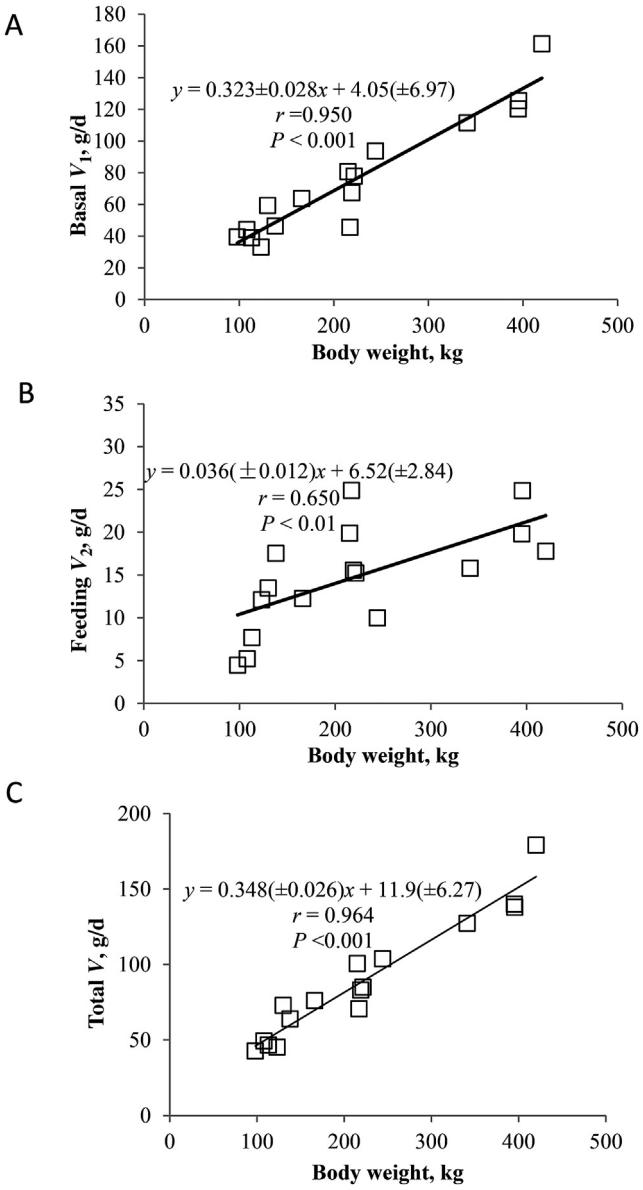

Fig. 5. Relationship between body weight (BW) and basal V_1 (A), feeding V_2 (B) and total V (C). The solid lines represent the best-fit regressions. V_1 = methane produced by residual substrate; V_2 = methane produced by newly ingested feed; V = methane emission.

production, which causes a shift in VFA production from acetate towards propionate, with accompanying changes of rumen microbial populations and great inefficiency of hydrogen mass transfer across the liquid–microbial interface, resulting in decreased and increased proportions of hydrogen sources and sinks, respectively (Leedle and Greening, 1988; Martin et al., 2010). Influx of great amounts of readily-degradable substrate also decrease ruminal pH, consequently inhibiting the growth and/or the activity of methanogens (Hegarty, 1999). In addition, in the current study the commercial concentrate mix also contained some fiber, with 415 g/kg NDF and 157 g/kg ADF, and these would contribute to basal V_1.

3.6. Dry matter intake, body weight and methane emissions

Feed intake is directly related to energy requirements by ruminants. Many predictive equations for methane emissions include DMI as a variable (Ellis et al., 2010; Ellis et al., 2009; IPCC, 2006). In our study, DMI and BW had a strong positive linear relationship ($P \leq 0.001$) with total V in dairy cows (Figs. 4 and 5). Although diet chemical composition can affect methane emissions from dairy cows (Bannink et al., 2011; Mills et al., 2001), DMI and BW were more important variables in our study, as they spanned a wider range (*i.e.*, DMI and BW ranges from 2.66 to 7.35 kg/d and from 98 to 420 kg, respectively) than differences in feed composition (*i.e.*, proportion of concentrate ranged from 0.539 to 0.788) in our data.

Feed intake is broadly assumed to scale to metabolic BW (Clauss et al., 2007; Demment and Vansoest, 1985), so that the DMI and BW were confounding variables that positively correlated with each other (data not shown). It is widely accepted that BW is an important variable that influences rumen–reticulum capacity (Van Soest, 1994; Weckerly, 2010). A larger BW needs higher absolute energy supply, so that rumen–reticulum capacity increases to meet heavier digesta loads (Smith and Baldwin, 1974; Van Soest, 1994), leading to more methanogens in basal digesta mass (Reynolds et al., 2004) and increased basal V_1. Goopy et al. (2014) reported low-methane yield sheep was associated with the smaller rumens and shorter rumen retention time. Figs. 4 and 5 showed that DMI and BW had a strong positive linear relationship ($P \leq 0.001$) with both basal V_1 and feeding V_2 in the non-lactating cows. This may be attributable to high intake animals having a larger digesta mass outflow from the rumen, with this needed to be balanced by a greater intake of new feed, leading to the increased feeding V_2.

4. Conclusion

The published models for estimating methane emissions are mainly based on feed intake and chemical composition of the diet (Bannink et al., 2011; Benchaar et al., 1998; Ellis et al., 2009), and rarely considered the diurnal pattern of methane emissions. These are affected by the feeding regime, and both feeding frequency and amount of feed offered can alter methane emissions (Beauchemin et al., 2008; Boadi et al., 2004; Janssen, 2010). The proposed model provides a means to quantify the sources of methane emissions when different feeding regimes are used, and thus estimate the potential of various strategies to limit total methane production. The model fits the kinetics of methane emission after feeding satisfactorily, and its parameters have acceptable accuracy to predict corresponding daily enteric methane emission. Importantly, the model identifies the contribution of total methane derived from both the residual substrate and newly ingested feed sources and this will then help predict the response to dietary interventions. Further studies are needed to quantify the parameters of this new model and identify factors that independently or in association impact on basal V_1 and feeding V_2.

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References

Aguerre MJ, Wattiaux MA, Powell JM, Broderick GA, Arndt C. Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide, and ammonia, lactation performance, and manure excretion. J Dairy Sci 2011;94:3081–93.

Bannink A, van Schijndel MW, Dijkstra J. A model of enteric fermentation in dairy cows to estimate methane emission for the Dutch National Inventory Report using the IPCC Tier 3 approach. Anim Feed Sci Technol 2011;166–67:603–18.
Beauchemin KA, Kreuzer M, O'Mara F, McAllister TA. Nutritional management for enteric methane abatement: a review. Aust J Exp Agric 2008;48:21–7.
Benchaar C, Rivest J, Pomar C, Chiquette J. Prediction of methane production from dairy cows using existing mechanistic models and regression equations. J Anim Sci 1998;76:617–27.
Bibby J, Toutenburg H. Prediction and improved estimation of linear models. London, UK: John Wiley & Sons; 1977.
Boadi D, Benchaar C, Chiquette J, Massé D. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. Can J Anim Sci 2004;84:319–35.
Chaves AV, Thompson LC, Iwaasa AD, Scott SL, Olson ME, Benchaar C, et al. Effect of pasture type (alfalfa vs. grass) on methane and carbon dioxide production by yearling beef heifers. Can J Anim Sci 2006;86:409–18.
Clauss M, Schwarm A, Ortmann S, Streich WJ, Hummel J. A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. Comp Biochem Physiol A Mol Integr Physiol 2007;148:249–65.
Demment MW, Vansoest PJ. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. Am Nat 1985;125:641–72.
Dhanoa MS, Lister SJ, France J, Barnes RJ. use of mean square prediction error analysis and reproducibility measures to study near infrared calibration equation performance. J Near Infrared Spectrosc 1999;7:133–43.
Dijkstra J, Neal H, Beever DE, France J. Simulation of nutrient digestion, absorption and outflow in the rumen – model description. J Nutr 1992;122:2239–56.
Ellis JL, Bannink A, France J, Kebreab E, Dijkstra J. Evaluation of enteric methane prediction equations for dairy cows used in whole farm models. Glob Change Biol 2010;16:3246–56.
Ellis JL, Dijkstra J, France J, Parsons AJ, Edwards GR, Rasmussen S, et al. Effect of high-sugar grasses on methane emissions simulated using a dynamic model. J Dairy Sci 2012;95:272–85.
Ellis JL, Kebreab E, Odongo NE, Beauchemin K, McGinn S, Nkrumah JD, et al. Modeling methane production from beef cattle using linear and nonlinear approaches. J Anim Sci 2009;87:1334–45.
Goopy JP, Donaldson A, Hegarty R, Vercoe PE, Haynes F, Barnett M, et al. Low-methane yield sheep have smaller rumens and shorter rumen retention time. Br J Nutr 2014;111:578–85.
Hegarty RS. Reducing rumen methane emissions through elimination of rumen protozoa. Aust J Agric Res 1999;50:1321–7.
Hindrichsen IK, Wettstein HR, Machmüller A, Jörg B, Kreuzer M. Effect of the carbohydrate composition of feed concentrates on methane emission from dairy cows and their slurry. Environ Monit Assess 2005;107:329–50.
IPCC. IPCC guidelines for national greenhouse gas inventories. Kanagawa, Japan: IGES, Hayama; 2006. Available: http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html.
Janssen PH. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim Feed Sci Technol 2010;160:1–22.
Johnson KA, Johnson DE. Methane emissions from cattle. J Anim Sci 1995;73:2483–92.
Khelaifia S, Raoult D, Drancourt M. A versatile medium for cultivating methanogenic archaea. PLoS One 2013:8.
Leedle JAZ, Greening RC. Postprandial changes in methanogenic and acidogenic bacteria in the rumens of steers fed high-forage or low-forage diets once daily. Appl Environ Microbiol 1988;54:502–6.
Lin LIK. A concordance correlation coefficient to evaluate reproducibility. Biometrics 1989;45:255–68.
Martin C, Morgavi DP, Doreau M. Methane mitigation in ruminants: from microbe to the farm scale. Animal 2010;4:351–65.
Mills JAN, Dijkstra J, Bannink A, Cammell SB, Kebreab E, France J. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: model development, evaluation, and application. J Anim Sci 2001;79:1584–97.
Moss AR, Newbold CJ, Givens DI. The impact of hexose partitioning in sheep in vivo. Proc Br Soc Anim Sci 2001;2001:157.
Ramin M, Huhtanen P. Development of equations for predicting methane emissions from ruminants. J Dairy Sci 2013;96:2476–93.
Reynolds CK, Durst B, Lupoli B, Humphries DJ, Beever DE. Visceral tissue mass and rumen volume in dairy cows during the transition from late gestation to early lactation. J Dairy Sci 2004;87:961–71.
Robinson JA, Strayer RF, Tiedje JM. Method for measuring dissolved hydrogen in anaerobic ecosystems – application to the rumen. Appl Environ Microbiol 1981;41:545–8.
Sakai S, Imachi H, Sekiguchi Y, Tseng IC, Ohashi A, Harada H, et al. Cultivation of methanogens under low-hydrogen conditions by using the coculture method. Appl Environ Microbiol 2009;75:4892–6.
Seo S, Tedeschi LO, Lanzas C, Schwab CG, Fox DG. Development and evaluation of empirical equations to predict feed passage rate in cattle. Anim Feed Sci Technol 2006;128:67–83.
Sherrod PH. NLREG, nonlinear regression analysis program. 1995. Brentwood, TN, USA.
Smith NE, Baldwin RL. Effects of breed, pregnancy, and lactation on weight of organs and tissues in dairy cattle. J Dairy Sci 1974;57:1055–60.
St-Pierre NR. Reassessment of biases in predicted nitrogen flows to the duodenum by NRC 2001. J Dairy Sci 2003;86:344–50.
Staerfl SM, Amelchanka SL, Kälber T, Soliva CR, Kreuzer M, Zeitz JO. Effect of feeding dried high-sugar ryegrass ('AberMagic') on methane and urinary nitrogen emissions of primiparous cows. Livest Sci 2012;150:293–301.
Tolkamp BJ, Schweitzer DPN, Kyriazakis I. The biologically relevant unit for the analysis of short-term feeding behavior of dairy cows. J Dairy Sci 2000;83:2057–68.
Van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Ithaca: Cornell University Press; 1994.
van Zijderveld SM, Gerrits WJJ, Apajalahti JA, Newbold JR, Dijkstra J, Leng RA, et al. Nitrate and sulfate: effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J Dairy Sci 2010;93:5856–66.
van Zijderveld SM, Gerrits WJJ, Dijkstra J, Newbold JR, Hulshof RBA, Perdok HB. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. J Dairy Sci 2011;94:4028–38.
Wang M, Janssen PH, Sun XZ, Muetzel S, Tavendale M, Tan ZL, et al. A mathematical model to describe *in vitro* kinetics of H_2 gas accumulation. Anim Feed Sci Technol 2013a;184:1–16.
Wang M, Sun XZ, Tang SX, Tan ZL, Pacheco D. Deriving fractional rate of degradation of logistic-exponential (LE) model to evaluate early *in vitro* fermentation. Animal 2013b;7:920–9.
Weckerly FW. Allometric scaling of rumen-reticulum capacity in white-tailed deer. J Zool 2010;280:41–8.