A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: model development, evaluation, and application

J. A. Mills, J. Dijkstra, A. Bannink, S. B. Cammell, E. Kebreab and J. France

J ANIM SCI 2001, 79:1584-1597.

A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation, and application[1]

J. A. N. Mills*,[2], J. Dijkstra†, A. Bannink‡, S. B. Cammell*, E. Kebreab*, and J. France*

*University of Reading, Centre for Dairy Research, Department of Agriculture, Earley Gate, P.O. Box 236, Reading RG6 6AT, U.K.; †WIAS Animal Nutrition, Wageningen University, Marijkeweg 40, P.O. Box 338, 6700 AH Wageningen, The Netherlands; and ‡Institute for Animal Science and Health, P.O. Box 160, NL-8200 AD Lelystad, The Netherlands

ABSTRACT: Dietary intervention to reduce methane emissions from lactating dairy cattle is both environmentally and nutritionally desirable due to the importance of methane as a causative agent in global warming and as a significant loss of feed energy. Reliable prediction systems for methane production over a range of dietary inputs could be used to develop novel dietary regimes for the limitation of feed energy loss to methane. This investigation builds on previous attempts at modeling methanogenesis and involves the development of a dynamic mechanistic model of whole-rumen function. The model incorporates modifications to certain ruminal fermentation parameters and the addition of a postruminal digestive element. Regression analysis showed good agreement between observed and predicted results for experimental data taken from the literature ($r^2 = 0.76$, root mean square prediction error = 15.4%). Evaluation of model predictions for experimental observations from five calorimetry studies (67 observations) with lactating dairy cows at the Centre for Dairy Research, in Reading, U.K., shows an underprediction (2.1 MJ/d) of methane production ($r^2 = 0.46$, root mean square prediction error = 12.4%). Application of the model to develop diets for minimizing methanogenesis indicated a need to limit the ratio of lipogenic to glucogenic VFA in the rumen and hindgut. This may be achieved by replacing soluble sugars in the concentrate with starch or substituting corn silage for grass silage. On a herd basis, the model predicted that increasing dietary energy intake per cow can minimize the annual loss of feed energy through methane production. The mechanistic model is a valuable tool for predicting methane emissions from dairy cows.

Key Words: Methane, Animal Models, Dairy Cows

 J. Anim. Sci. 2001. 79:1584–1597

Introduction

In 1997, international action was taken in the Kyoto Protocol to focus on the stabilization of atmospheric levels of six greenhouse gases, including methane (Morard, 1999). Methane, as a contributor to global warming, is second only to carbon dioxide. The majority of methane production from livestock is associated with ruminants. Indeed, cattle account for about 73% of the 80 Tg (1 Tg = 1 million metric tons) produced by livestock each year (Gibbs and Johnson, 1994). Methanogenesis also represents a loss of feed energy, and the potential to increase the ME value of dairy cow diets through a reduction in methane production is significant.

Calorimetry studies undertaken at the Centre for Dairy Research (**CEDAR**, Reading, U.K.) have produced a database containing simultaneous measurement of methane emissions and dietary inputs for dairy cows. This investigation focuses on the ability of a modified version of the Dijkstra et al. (1992) mechanistic rumen model to quantify methane production from lactating dairy cows. The first objective was to construct a methane module within the existing rumen model that allows prediction of methane emissions over a range of dietary inputs. The second objective was to apply the model in the development of novel dietary strategies for reducing methane emissions.

Materials and Methods

Model Development

Recently, Benchaar et al. (1998b) used both empirical regression equations and mechanistic approaches to

[1]Financial support provided by MAFF Environment [CC0239] is appreciated by the authors. The research of Jan Dijkstra has been made possible by a fellowship from the Royal Netherlands Academy of Arts and Sciences.

[2]Correspondence: phone: 44 (0)118 9316783; E-mail: j.a.n.mills@reading.ac.uk.

Received August 8, 2000.
Accepted January 8, 2001.

simulate methane emissions by dairy cows. Two dynamic mechanistic models of ruminant digestion (Baldwin et al. 1987; Dijkstra et al., 1992) were evaluated alongside the regression equations for their ability to predict published values of methane emissions. The model of whole-rumen function described by Dijkstra et al. (1992) existed only on the basis of carbon and nitrogen fluxes. Therefore, it was modified to incorporate the framework for methane production already present within the Baldwin et al. (1987) model. Benchaar et al. (1998b) showed an improved prediction of methane production with both mechanistic models in comparison with regression equations. However, Benchaar et al. (1998b) presented the Dijkstra et al. (1992) model as the most reliable model for simulating methane production over the range of diets tested, with the model of Baldwin et al. (1987) displaying a larger error of prediction (36.93% vs 19.87% of the observed mean).

In a comment on the investigation by Benchaar et al. (1998b), Donovan and Baldwin (1998) suggested that there was no systematic error of prediction for methane emissions when using the Baldwin et al. (1987) model. Donovan and Baldwin (1998) suggest that the use of incorrect input parameters within the study by Benchaar et al. (1998b) was a major source of error. Indeed, Donovan and Baldwin (1998) demonstrated a tendency for slight underprediction of methane emissions in comparison to a significant overprediction shown by Benchaar et al. (1998b). Benchaar et al. (1998a) suggest that the conflicting results are primarily a result of uncertain input parameters within the mechanistic approach to rumen modeling.

Further improvement of the representation of methanogenesis within the Dijkstra et al. (1992) model may be possible. In particular, and as acknowledged by Benchaar et al. (1998b), the use of the Dijkstra et al. (1992) rumen model to predict experimental observations of whole-animal methane production does not account for postruminal fermentation. In sheep, 8 to 16% of total methane emissions may result from fermentation within the cecum and colon (Murray et al., 1976; Murray et al., 1978). Failure to account for hindgut methanogenesis might account for underestimation by existing models.

A revised representation of methanogenesis designed specifically for incorporation into the Dijkstra et al. (1992) rumen model is described below, followed by a mechanistic framework for the simulation of postruminal fermentation. Unless otherwise stated, the structure of the rumen model is as presented by Dijkstra et al. (1992). The general framework for predicting methane production is a modification of that described by Baldwin (1995). Excess hydrogen produced during fermentation is partitioned between its use for microbial growth, biohydrogenation of unsaturated fatty acids, and the production of glucogenic VFA. The assumption is made that the remaining hydrogen is used solely and completely for methanogenesis. This approach treats hydrogen as a zero pool within the model (France et al., 1992).

The Production of Hydrogen During Fermentation. Calculation of hydrogen produced during the production of the lipogenic VFA, acetate and butyrate, following the fermentation of carbohydrate and protein in the rumen (PHyferm) is as follows:

$$\text{PHyferm (mol } H_2\text{/d)} = (\text{PAc} \times 2) + (\text{PBu} \times 2)$$

where PAc and PBu are the amounts (mol/d) of acetate and butyrate produced during fermentation. Therefore, 2 mol of H_2 are produced per mole of acetate or butyrate (Baldwin, 1995).

Further hydrogen is produced as the microbial population utilizes amino acids rather than nonprotein nitrogen (**NPN**) for growth (PHyMg). In using the representation of methanogenesis described by Baldwin et al. (1987), Benchaar et al. (1998b) assumed parameter values for hydrogen flux during microbial growth that are similar to those presented by Baldwin et al. (1987). However, the microbial growth calculations within the Dijkstra et al. (1992) rumen model should be considered in terms of polysaccharide-free microbial dry matter. For this reason, the constants for hydrogen flux during microbial growth on preformed amino acids or NPN were reevaluated using the balance equations presented by Reichl and Baldwin (1975) and Baldwin (1995). During these calculations, the chemical composition of the microbial matter was assumed to be as presented in table 4 of Dijkstra et al. (1992). The revised requirement for hydrogen during microbial growth without preformed amino acids was 0.41 mol hydrogen per kilogram of microbes in comparison to 2.71 mol/kg, as used by Benchaar et al. (1998b). During growth on amino acids, there is a net production of 0.58 mol hydrogen per kilogram microbes in comparison to 0.42 mol/kg as used by Benchaar et al. (1998b); therefore

$$\text{PHyMg (mol } H_2\text{/d)} = \text{Mgaa} \times 0.58$$

where Mgaa is the quantity of microbial matter (kg DM/d) produced from growth on amino acids and 0.58 is the yield of H_2 per gram of microbial matter.

The Utilization of Hydrogen During Fermentation. The calculation of hydrogen utilization during the production of the glucogenic VFA, propionate and valerate, following the fermentation of carbohydrate and protein in the rumen (UHyferm) is as follows:

$$\text{UHyferm (mol } H_2\text{/d)} = \text{PPr} + \text{PVl}$$

where PPr and PVl are the amounts (mol/d) of propionate and valerate produced during fermentation. Therefore, there is a net utilization of 1 mol H_2/mol of propionate and valerate (Baldwin, 1995). The calculation of hydrogen utilization following growth of microbes on NPN (UHyMg) is as follows:

$$\text{UHyMg (mol } H_2\text{/d)} = \text{MgNPN} \times 0.41$$

where MgNPN is the quantity of microbial matter (kg/d) produced from growth on NPN and 0.41 is the requirement for H_2 per gram of microbial matter (see hydrogen production section). The calculation of hydrogen utilization following the biohydrogenation of ingested lipid (UHyLi) is done with the equation

$$\text{UHyLi (mol } H_2\text{/d)} = \text{PLi} \times \text{Liferm} \times 1.805 \times 2.0$$

where PLi is the intake of feed lipid (mol/d), Liferm is the proportion of feed lipid subject to lipolysis within the rumen, 1.805 is the coefficient describing the moles of unsaturated fatty acid per mole of feed lipid, and 2.0 is the moles of hydrogen utilized per mole of unsaturated fatty acid (Baldwin, 1995).

Hydrogen Balance and Methane Yield in the Rumen. The excess hydrogen available for methanogenesis (Hy) is calculated as

$$\text{Hy (mol } H_2\text{/d)} = \text{PHyferm} + \text{PHyMg} - \text{UHyferm} - \text{UHyMg} - \text{UHyLi}$$

Ruminal methane production is calculated using the equation

$$\text{PCH}_4\text{ (mol/d)} = \text{Hy} / 4.0$$

where 4.0 is the moles of H_2 required for the production of 1 mol of methane resulting from the reduction of CO_2. The calculation of methane energy is made with

$$\text{PCH}_4\text{ (MJ/d)} = \text{PCH}_4\text{ (mol/d)} \times 0.883$$

where 0.833 is the GE of methane in megajoules per mole.

Additional Dietary Inputs. The Dijkstra et al. (1992) rumen model ignores the contribution of glycerol from the lipolysis of dietary trigylceride in the fermentation process. However, in order to maintain a hydrogen balance within the model, the glycerol is assumed to enter the amylolytic hexose pool. Pectins are assumed to enter the soluble carbohydrate pool.

New Stoichiometry. Due to the association between VFA production and hydrogen metabolism in the rumen, accurate simulation of VFA molar proportions is needed for reliable methane prediction. In the original model (Dijkstra et al., 1992), the molar proportions of rumen VFA were based on the stoichiometric data of Murphy et al. (1982). However, there is a need for refinement of these coefficients (Bannink et al., 1997). Bannink et al. (2000) have recently developed a new stoichiometry for fermentation within the rumen based entirely on experimental observations with lactating dairy cows. Therefore, the rumen model of Dijkstra et al. (1992) has been modified to accommodate these stoichiometric coefficients.

Modification to Rumen pH Parameters. The original rumen model (Dijkstra et al., 1992) requires the mean rumen pH to be specified as an input, based on data reported in the literature. In conjunction with the mean pH value, the minimum daily pH and time below a critical pH (pH 6.3) are required due to the influence of these parameters on absorptive processes, fiber degradation, and microbial recycling within the rumen. Bannink et al. (1997) described these pH values as uncertain inputs within the model and showed significant variation in duodenal NDF flow following a sensitivity analysis that involved manipulation of these parameters within acceptable limits. For the purposes of this investigation, the model has been modified to allow a more dynamic determination of pH based on the relationship between VFA concentration and pH described by Tamminga and Van Vuuren (1988) ($r^2 = 0.71$).

The following calculates the mean daily rumen pH (Tamminga and Van Vuuren, 1988):

$$\text{pH} = 7.73 - (0.014 \times \text{cVFA})$$

where cVFA is the concentration of VFA in the rumen (m*M*). For simplicity in the current modeling exercise, the minimum daily pH (PM) is subsequently estimated as mean pH minus 5%, although experimental observations show a range of 1 to 15% of mean pH depending on feeding frequency (Sutton et al., 1986):

$$\text{PM} = \text{pH} - (\text{pH} \times 0.05)$$

where PM is the minimum pH reached during the day. Time below a critical pH for reduced fiber digestion (TF h/24 h), pH 6.3 (Erdman, 1988) is calculated as

$$\begin{aligned} \text{TF (h/24 h)} &= (-10.59 \times \text{pH}) + 76.82 \\ \text{TF (h/24 h)} &= 0 \qquad \text{if pH} \geq 7.2 \\ \text{TF (h/24 h)} &= 24 \qquad \text{if pH} \leq 5.0 \end{aligned}$$

This relationship yields a linear increase in TF (h/24 h) from 0 at pH 7.2 and above to 24 at pH 5.0 and below. This assumes a twice-daily feeding pattern (Sutton et al., 1986).

Modeling Postruminal Digestion and Fermentation. To account for postruminal methanogenesis within the model, the assumption was made that the fermentation process is similar to that occurring in the rumen. Therefore, a mechanistic model of large intestinal fermentation has been constructed using the existing rumen model as a basis, and the following modifications were made. Inputs to the large intestine are rumen outflows modified for small intestinal digestion. The capacity for small intestinal digestion of starch is assumed to depend on the degradability and quantity of starch flowing from the rumen (Nocek and Tamminga, 1991; Mills et al., 1999b). Therefore, the following relationship described by Nocek and Tamminga (1991) has been used to produce a coefficient for small intestinal starch digestibility (SdSi):

$$\text{SdSi (\%)} = -0.728 \times \text{RES} + 87.9$$

where RES (% starch intake) is rumen escape starch and includes microbial storage polysaccharides.

The digestibility of microbial amino acids is set at 0.75. This value is lower than that estimated by AFRC (1993) for postruminal disappearance to allow for degradation in the large intestine. The true digestibility of undegraded but potentially rumen-degradable feed protein is set at 0.7 (Palmquist et al., 1993), but the indigestible protein fraction remains undegraded. The NDF is assumed to remain undegraded in the small intestine. The digestibility of feed and microbial lipid is set at 0.9 (Palmquist et al., 1993) to allow for calculation of fecal GE. However, lipid metabolism in the large intestine is ignored due to its limited significance for methanogenesis following saturation of fatty acids in the rumen. Hexose escaping rumen fermentation is completely absorbed within the small intestine.

Quantitative data regarding the passage of digesta through the large intestine in lactating dairy cows are extremely limited in availability. The passage of digesta is a combination of plug flow and mixing (France et al., 1993). However, because the large intestinal model is based around that of the rumen, the passage kinetics are assumed to be those of a system involving complete mixing of digesta. The data from the literature suggest a mean retention time (MRT) of approximately 9 to 13 h (Huhtanen and Vanhatalo, 1997; Vanhatalo and Ketoja, 1995; Pellikaan et al., 1999). This equates to a fractional passage rate range of 7.7 to 11.1%/h with increases associated with increasing DMI. Therefore, fractional passage rate in the large intestine is calculated in the model using the following equation:

$$\text{KpaLi (\%/h)} = 1/(-0.2 \times \text{DMI} + 13)$$

where DMI is in kilograms per day. This equation yields a linear increase in fractional passage rate from 9%/h at DMI of 10 kg to 11%/h at 20 kg DMI/d. Some selective retention of microbial matter within the cecum (Van Soest, 1994) is assumed, and microbial passage rates are set at 0.85 of the digesta passage rate.

For the sake of the modeling exercise, protozoa are assumed absent from the large intestine (Demeyer, 1991). Large intestinal volume is set at 10% of calculated rumen volume (Parra, 1978).

Dietary Inputs. Where possible during evaluation of the model, nutritional inputs are derived from analytical data reported within the experiments. However, where information is lacking, estimation of certain inputs was made according to data published elsewhere in the literature. For example, the description of the in situ kinetics of feed carbohydrate and protein, the data presented by Nocek and Grant (1987), Tamminga et al. (1990); Van Vuuren et al. (1990), Nocek and Tamminga (1991), Bosch et al. (1992), and Huhtanen and Vanhatalo (1997) have been used.

Model Summary. The differential equations of the 34 state variables, representing the nutrient and microbial pools in the rumen and hindgut, are integrated numerically for a given set of initial conditions and parameter values. The model was written in the Advanced Continuous Simulation Language (ACSL, 1995). A fourth-order fixed-step-length Runge-Kutta method with an integration interval of 0.0001 d was used. The results presented were obtained by running the model until a steady state was achieved.

Model Application

The model was used to simulate digestion and determine the efficacy of a range of dietary strategies designed to minimize whole-tract methanogenesis. These strategies were evaluated through examination of methane emissions as well as digestibility of the major nutrients and the overall diet metabolizability (ME divided by GE; **ME/GE**). Digestible energy was simulated in the model by deducting the calculated GE of the feces from the calculated GE of the feed. Simulated methane and urinary energy values were then subtracted from the DE to yield a calculated ME value for each diet. Urine energy values were calculated using an empirical model of urinary excretion combined with the assumption that N excreted in the urine was related to GE. This was described by the following locally derived linear relationship:

$$\text{Urine energy (MJ/d)} = 49.461(\text{Urine N, g/d}) + 1.695$$
$$r_2 = 0.86$$

Nitrogen excretion in the urine was related to nitrogen intake using the exponential relationship described by Castillo et al. (1999). The methods of dietary intervention for reducing methanogenesis simulated by the model were increasing DMI, replacing sugar in the concentrate with starch, and increasing dietary energy density through supplementation of grass silage diets.

Model Evaluation

Evaluation of the model was initially performed on the same data set used by Benchaar et al. (1998b). Subsequently, data concerning whole-animal methane emissions from lactating dairy cows involved in calorimetry studies at CEDAR were used to compare model predictions with experimental observations. Data from five calorimetry studies, representing a range of nutritional strategies involving lactating dairy cows, were used to test model predictions. Animal and diet details are summarized in Table 1. Unless otherwise stated, the trials involved twice-daily feeding and milking. Cows were trained to use respiration chambers before the experiments began.

In Trial 1, cows were fed a forage mixture of 3:1 (DM basis) grass silage:corn silage. This forage was fed at a 1:1 (DM basis) ratio with either a high- or low-starch

Table 1. Trial summary for CEDAR[a] calorimetry studies

Trial	No. of cows	No. of observations	Mean BW, kg	DMI, kg/d[b]	Milk yield, kg/d
1	6	24	600	18.4	26.9
2	4	16	620	19.8	27.0
3	4	7	606	16.3	29.7
4	4	8	671	17.7	21.7
5	3	12	651	20.9	31.1

[a]Centre for Dairy Research.
[b]DMI corrected for volatile losses.

concentrate as part of a total mixed ration (**TMR**) and at ad libitum or a restricted level of intake (85% of ad libitum DM). Trial 2 involved the feeding of a 1:1 (DM basis) mixture of high- and low-DM whole-crop wheat (**WCW**) silage as described by Sutton et al. (2001). The WCW mix was fed with first-cut grass silage in a 1:2 (DM basis) grass silage:WCW ratio. The forage was offered for ad libitum consumption with 8.2 kg DM of a concentrate mixture. Treatments involved the replacement of WCW silage with NaOH treated WCW silage or by altering the formulation of the concentrates. Trials 3 and 4 involved feeding fresh grass three times daily ad libitum. Concentrates were fed at 5.2kg DM/d. Trial 4 was undertaken 12 mo after Trial 3. Trial 5 involved feeding a 3:1 (DM basis) corn silage:grass silage mixture with experimental procedures presented by Cammell et al. (2000). The corn silage was harvested at two stages of maturity, defined by DM content. Four different concentrates were fed alongside both forage mixtures. Concentrates varied in starch source and degradability and were fed at 8.7 kg DM/d with the forage mixture at ad libitum intake.

Statistics. Regression analysis between observed and predicted values has been used to demonstrate the reliability of model predictions. Error of prediction is estimated from the calculation of root mean square prediction error (**MSPE**) and expressed as a percentage of the observed mean:

$$\text{MSPE} = \Sigma\ (O_i - P_i)^2/n$$

where i = 1, 2, . . . n; n is the number of experimental observations; and O_i and P_i are the observed and predicted values (Bibby and Toutenburg, 1977). The MSPE is decomposed into overall bias of prediction, deviation of regression slope from 1, and the disturbance proportion. These components of MSPE were defined by Benchaar et al. (1998b).

Results

Model Prediction of Literature Data

Results of the regression between observed and predicted methane production from the literature experiments are shown in Figure 1. In this instance, the model overestimated average methane production by 0.52 MJ/d. The r^2 was 0.76 and the root MSPE was 15.4% of the observed mean. The contribution of the disturbance proportion was 73% of the MSPE. For all simulations, the mean (and SD) simulated contribution of large intestinal fermentation to total methane emissions was 9.14% ± 2.64.

Model Prediction of CEDAR Trials

The results from one cow (Cow 28, Trial 5) have been removed from the analysis due to unusually low nutrient digestibilities and methane emissions compared with contemparies on the same dietary regime. Figure 2 displays the regression analyses between methane production for all five trials (67 observations). Table 2 displays the summary statistics for each of the 5 trials, together with a summary for all observations combined. Overall mean predicted methane production was underestimated by 2.09 MJ/d with an r^2 of 0.46 and a root MSPE of 12.36% of the observed mean. The slope and intercept were different from 1 ($P < 0.05$) and 0 ($P < 0.05$) respectively. The decomposition of MSPE showed a bias of prediction with a tendency to underestimate methanogenesis as observed methane emissions increased. However, when the individual trial results were considered, only Trials 1 and 2 showed a significant bias ($P < 0.05$). In contrast, the r^2 was greater for Trials 1, 2 and 5 than for Trials 3 and 4 (Table 2). The unexplained error due to the disturbance proportion was 47% of MSPE for all trials combined. The mean contribution of large intestinal fermentation to total methanogenesis was 9.1% (± 0.81 SD). Due to the underestimation of mean total methane production, simulated loss of GE through methanogenesis was 0.59% lower than observed (6.24% ± 0.6 SD vs 6.83 ± 0.63 SD).

Table 3 summarizes the observed and predicted digestibilities for the principal dietary nutrients. The model gave a reliable prediction of NDF and protein digestibility with a root MSPE of 6.2% and 7.15% respectively. There was also a low root MSPE (6.55% of observed mean) for total-tract starch digestibility. However, mean predicted starch digestibility was overestimated for Trials 1 (97.2% vs 91.7%) and 2 (97.8% vs 88.8%).

Table 4 displays the mean simulated fate of hydrogen in the rumen and large intestine. In the rumen, methane was the major hydrogen sink (78.2% ± 1.31 SD) for all diets. The production of the glucogenic VFA was the

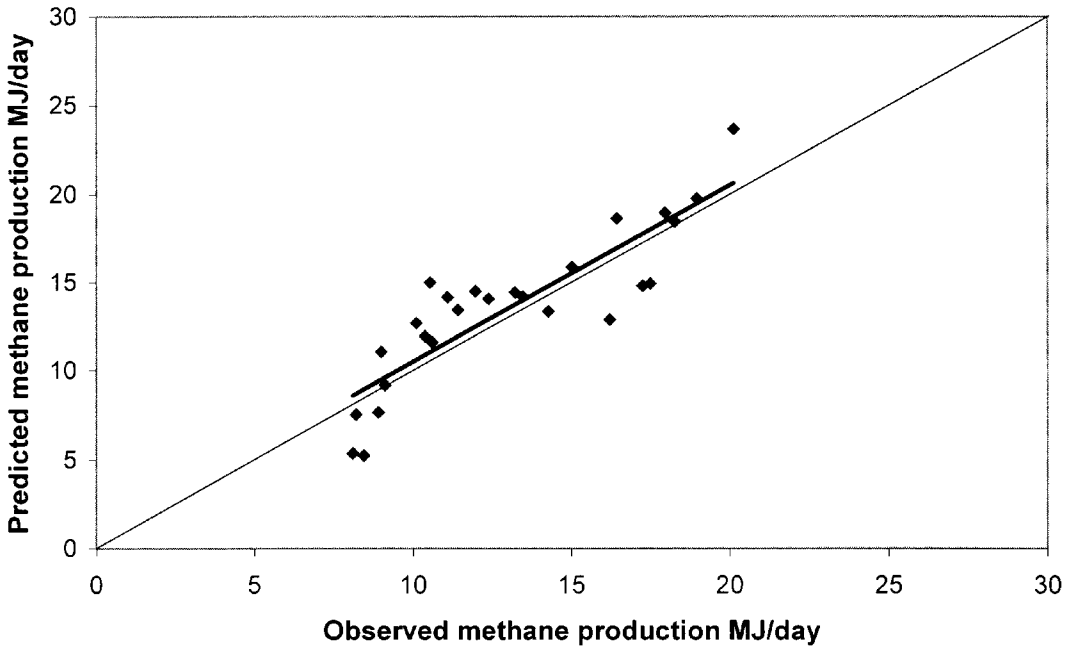

Figure 1. Observed vs predicted methane production using the current model to simulate experiments described in the literature. Mean predicted methane production (MJ/d) = 13.67; root mean square prediction error (%) = 15.39; bias proportion (%) = 5.99; error due to regression (%) = 24.19; disturbance proportion (%) = 73.31. Line of equality (————); Regression line (————) y = 1.0 (± 0.13)x + 0.49 (± 0.51) r^2 = 0.76. Data are from Belyea et al. (1985); Coppock et al. (1964); Moe and Tyrrell, (1972, 1973, 1977, 1979a,b); Tyrrell and Moe, (1972); Moe et al. (1973); Holter et al. (1986, 1990); Harlan et al. (1991); Holter and Young, (1992); Cushnahan et al. (1995).

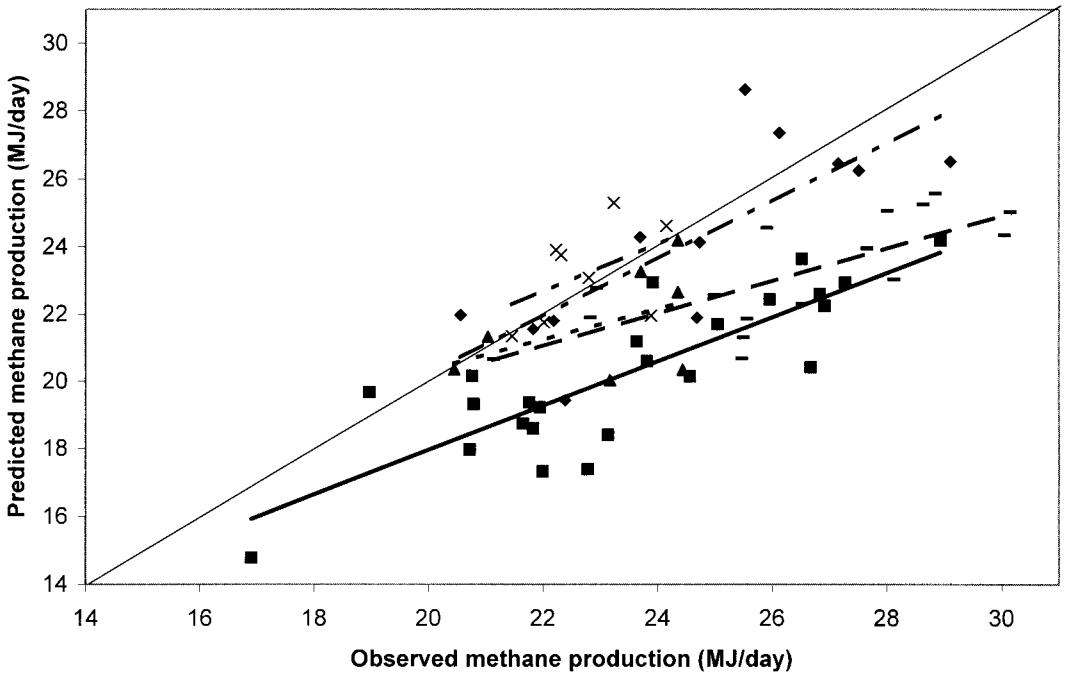

Figure 2. Observed vs predicted methane production for Centre for Dairy Research calorimetry trials. Summary statistics are shown in Table 2. ■ ———— Trial 1, – – – Trial 2, ▲- - - - - Trial 3, x—·-·— Trial 4, ◆—·-·-·— Trial 5. Trial 1 y = 0.66 (± 0.09)x + 4.81 (± 2.19) r^2 = 0.7; Trial 2 y = 0.49 (± 0.11)x + 10.33 (± 2.92) r^2 = 0.58; Trial 3 y = 0.46 (± 0.39)x + 11.04 (± 8.97) r^2 = 0.22; Trial 4 y = 0.72 (± 0.55)x + 6.88 (± 12.43) r^2 = 0.22; Trial 5 y = 0.86 (± 0.22)x + 3.09 (± 5.42) r^2 = 0.61.

Table 2. Summary of observed vs predicted methane production of five CEDAR[a] trials[b]

Item	Methane production, MJ/d		Root MSPE, %[c]	ECT[d]	ER[e]	ED[f]	r^2	CH_4 % LI[gh]
	Observed[h]	Predicted[h]						
Trial 1	23.5 ± 0.6	20.3 ± 0.5	15.3	10.41	0.02	2.6	0.70	9.3 ± 0.2
Trial 2	26.4 ± 0.7	23.2 ± 0.4	13.7	10.34	0.11	2.86	0.58	9.3 ± 0.1
Trial 3	23.1 ± 0.6	21.7 ± 0.6	8.9	1.78	0.73	2.15	0.22	8.4 ± 0.1
Trial 4	22.8 ± 0.3	23.2 ± 0.5	6.0	0.20	0.96	0.69	0.22	8.1 ± 0.1
Trial 5	24.6 ± 0.8	24.2 ± 0.8	7.4	0.19	0.7	2.66	0.61	9.4 ± 0.2
All Trials	24.3 ± 0.3	22.2 ± 0.3	12.4	4.36	0.5	4.19	0.46	9.1 ± 0.1

[a]Centre for Dairy Research.
[b]Regressions shown in Figure 2.
[c]Root mean square prediction error expressed as a percentage of the observed mean.
[d]Error due to bias.
[e]Error due to regression.
[f]Error due to disturbance.
[g]Percentage of total methane produced in large intestine.
[h]Mean ± SEM.

next largest sink (18.5% ± 1.28 SD) with fatty acid hydrogenation (2.6% ± 0.49 SD) and microbial growth (0.6% ± 0.04 SD) forming considerably smaller hydrogen sinks.

Discussion

Model Application

Increasing DM Intake. It is generally accepted that, as DMI increases, the total methane production also increases (Kriss, 1930; Axelsson, 1949; Wilkerson et al., 1995). However, the proportion of ingested energy lost as CH_4 may decline as intake increases. Figure 3 shows the relationship between DMI and methane emissions as predicted by the model. This simulation involved a constant dietary composition across all intakes. The proportion of GE lost as CH_4, declined in a linear manner from 6.6% to 6.0% as intake increased from 10 to 25 kg DM/d. An implication of this relationship is that extensive systems of milk production, involving the use of more cows fed a restricted intake, tend to lose more feed energy as methane while producing similar total milk production from the farm unit as a whole. Table 5 compares the two production systems for a dairy herd with a daily milk output of 2,000 kg. In this simulation, cows that were fed a restricted amount (13.5 kg/d) produced over 20% more methane per lactation than those cows that consumed ad libitum (17.5 kg/d). This was despite only a 0.25% difference in the partitioning of GE toward methane between the two production systems. Methane production per kilogram of milk produced was 0.79 and 0.66 MJ/kg for the restricted vs ad libitum diets, respectively.

Supplementation of Grass Silage Based Diets. The traditional approach to the winter feeding of dairy cows in the United Kingdom has revolved around the use of grass silage as the principal forage and sometimes the sole dietary component. Supplementation of these diets with concentrates is common; more recently, there has been a move toward including corn silage as a major forage component. The model has been used to simulate the supplementation of a basic grass silage diet (10 kg DM/d) with either more grass silage (increasing DMI), corn silage, or a general-purpose concentrate mixture. Each supplement was fed up to 7 kg DM/d in addition to the 10 kg of grass silage DM. Simulated ME intake ranged from 115 MJ/d to over 200 MJ/d for the corn and concentrate diets and up to 190 MJ/d for the 100% grass silage diet. As demonstrated previously, the pro-

Table 3. Summary of observed vs predicted total-tract nutrient digestibilities of five CEDAR[a] trials

Item	Observed[b]	Predicted[b]	Root MSPE, %[c]	ECT[d]	ER[e]	ED[f]	r^2
NDF	62.4 ± 0.9	64.3 ± 1.1	6.20	3.67	4.11	7.35	0.86
Starch	93.6 ± 0.6	97.5 ± 0.1	6.55	15.2	0.93	18.7	0.11
Nitrogen	69.4 ± 0.7	70.3 ± 0.2	7.15	0.73	0.41	23.8	0.20

[a]Centre for Dairy Research.
[b]Mean ± SEM.
[c]Root mean square prediction error expressed as a percentage of the observed mean.
[d]Error due to bias.
[e]Error due to regression.
[f]Error due to disturbance.

Table 4. Simulated fate of hydrogen in the rumen and large intestine for all five CEDAR[a] trials

Item	Rumen[b]	Large intestine[b]
	% of total H_2 utilization	
VFA	18.5 ± 0.2	19.9 ± 0.03
Microbes	0.6 ± 0.005	0.8 ± 0.003
Biohydrogenation	2.6 ± 0.06	NA
Methane	78.2 ± 0.2	79.3 ± 0.04

[a]Centre for Dairy Research.
[b]Mean ± SEM.

Table 5. Simulated whole-herd methane emissions for cows fed at restricted intake or consuming ad libitum over one lactation period

	Restricted intake	Ad libitum
Milk yield, kg/d	20	30
305-d yield, kg/d	6,100	9,150
Milk yield (herd basis), kg/d	2,000	2,000
Daily ME requirement[a] (per cow)		
Maintenance, MJ/d	65	65
Milk, MJ/d	58	87
kl[b]	0.64	0.64
Total ME requirement, MJ/d	156	201
GE concentration in DM, MJ/kg	18	18
ME concentration in DM, MJ/kg	11.5	11.5
DM intake required, kg/d	13.53	17.47
Methane emissions		
GE, % as CH_4	6.5	6.25
CH_4, MJ·cow^{-1}d·d^{-1}	15.8	19.7
CH_4, MJ·herd^{-1}d·d^{-1}	1,583	1,310
CH_4, GJ·herd^{-1}·lactation^{-1}	483	400

[a]Assumes no BW change and no fetus.
Efficiency of utilization of ME for lactation.

portion of GE lost as methane declined with increasing DMI for all diets. However, the decline was slowest for the concentrate diet and fastest for the diet with supplemental corn silage (Figure 4). Figure 5 shows that when the efficiency of ME supply from feed energy was considered, the addition of concentrate provided the greatest improvements. However, in terms of the balance achieved between increased ME supply and the reduction in methane production, supplementation with corn silage was the most efficient of all the diets fed (Figure 6). Because ME is an improved indicator of energy available to the cow in comparison with GE, Figure 6 would suggest that, of the strategies tested, the best method for increasing the energy supply to dairy cows while minimizing methane production was through the substitution of corn silage for grass silage.

Changing Concentrate Energy Source. Soluble sugars can feature as a major constituent of the concentrate fraction in which feedstuffs such as molasses or sugar beet and citrus pulps are included. These sugars are fermented rapidly in the rumen to produce mainly acetate and butyrate (Van Soest, 1994). However, the ratio of lipogenic to glucogenic VFA produced from starch fermentation is considerably less on both forage- and concentrate-based diets (Murphy et al., 1982; Bannink et al., 2000). Sugars are also more completely degraded within the rumen than starch. Therefore, as starch replaces sugar in the concentrate, total methane emissions should decline. When the model was used to simulate a range of concentrate compositions varying in starch:sugar ratio from 0 to 100% starch, the total CH_4 production decreased by 14.7%. Because GE intake was

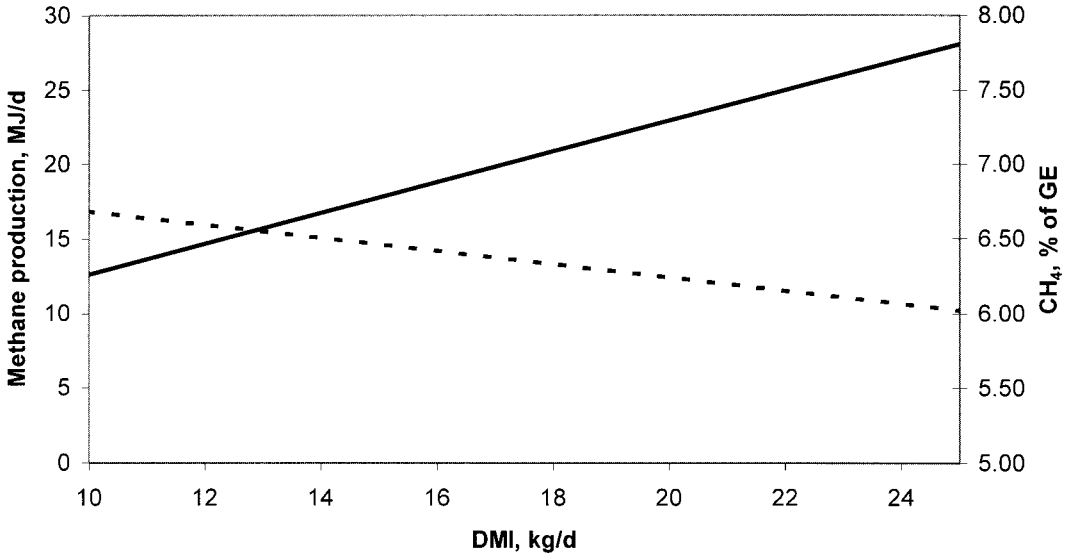

Figure 3. Simulated methane emissions and methane output as a percentage of GE intake from a lactating dairy cow fed increasing levels of DMI. Diet consists of 50:50 grass silage:concentrate. Methane production (———); methane as % GE (- - - - -).

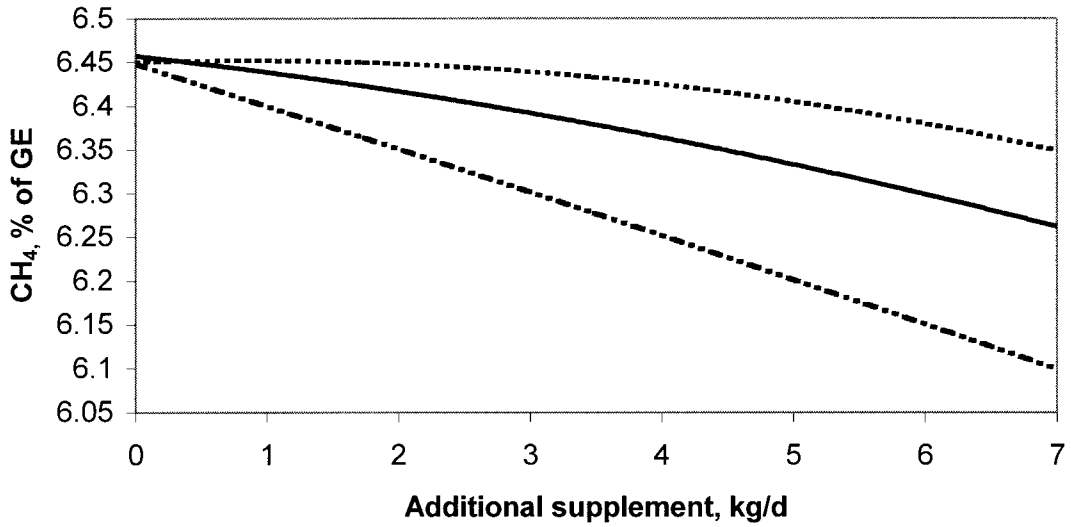

Figure 4. Simulated methane emissions as a percentage of GE intake in lactating dairy cows fed either additional grass silage, concentrate, or corn silage to a grass silage-based diet. Grass silage (———); grass silage + concentrate (·····); grass silage + corn silage (— · —).

constant for each diet fed, the proportion of GE lost as methane also declined in a linear manner (Figure 7). When the ratio of starch to sugar increased from 30 to 100% starch, ME/GE increased gradually by 0.23% for every 10% increase in starch inclusion. When sugar increased from 70 to 100% of the total inclusion, there was a rapid decline in ME/GE of the total diet at a rate of 1.2% for every 10% decline in starch inclusion.

Mainly forage diets are often supplemented with sugar-based concentrates to provide a rapidly available source of energy for the rumen microbes or to increase the palatability of the diet DM and hence stimulate intake. The results of the simulation (Figure 7) suggest that the substitution of the sugar with a rapidly fermentable starch source (wheat or barley) would not only reduce CH_4 emissions, but also yield a considerable energetic benefit to the dairy cow due to the elevation in ME/GE.

When cornstarch was used to replace the sugar completely, the total-tract starch digestibility declined by 2.2%. However, the reduced starch fermentation in the rumen elevated total-tract NDF digestibility by 1.2%. Utilizing cornstarch increased the proportion of total-tract starch digestion occurring in the small intestine

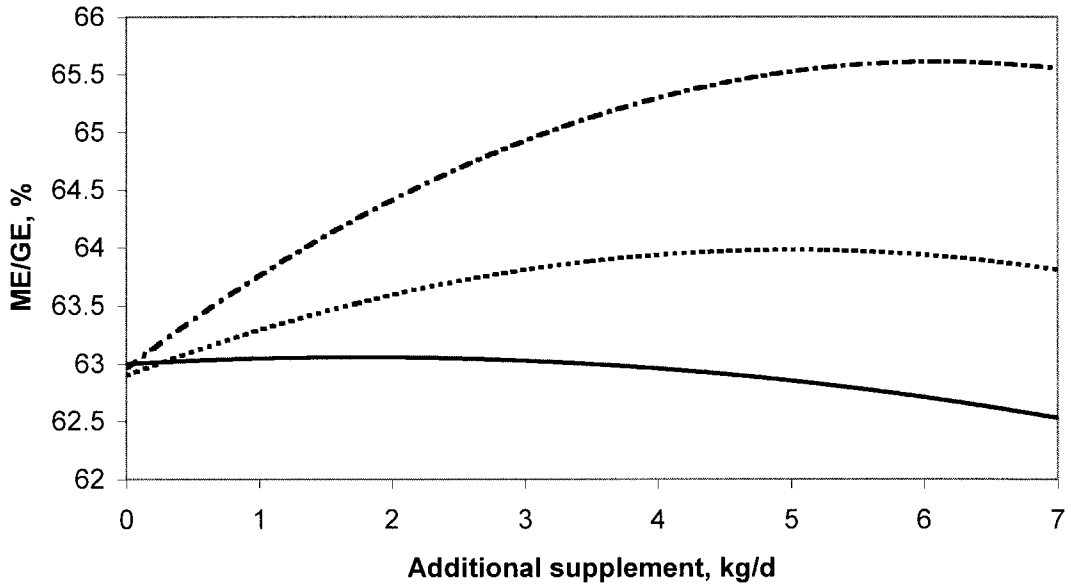

Figure 5. Simulated change in ME/GE for lactating dairy cows fed additional grass silage, concentrate or corn silage to a grass silage-based diet. Grass silage (———); grass silage + concentrate (·····); Grass silage + maize silage (— · —).

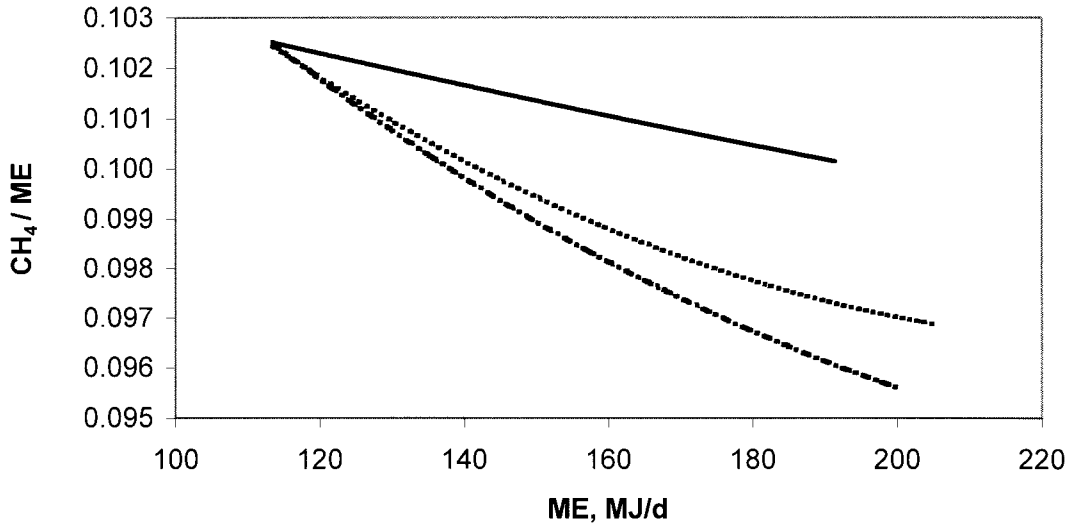

Figure 6. Simulated CH_4 energy as a proportion of ME available to lactating dairy cows fed additional grass silage, concentrate or corn silage on a grass silage-based diet. Grass silage (———); grass silage + concentrate (▪▪▪▪▪); grass silage + maize silage (—▪—).

(22%) compared with wheat starch (17%). The decreased digestibility also contributed to a decreased methane production. The combination of these digestive events leads to an ME/GE that is elevated to an even greater degree than that for the inclusion of the more rapidly degradable starch. The total decline in CH_4 production was marginally greater for cornstarch in comparison to wheat starch. Because the energy available to the cow was similar in both instances, the use of cornstarch may be the more environmentally efficient dietary strategy.

General Discussion

When Benchaar et al. (1998b) used the Dijkstra et al. (1992) model for a similar data set, the model under predicted methane emissions by 1.26 MJ/d with an r^2 of 0.71 and a root MSPE of 19.87%. The increase in simulated methane emissions observed in this investigation is to be expected following the modification of the model. The new hydrogen production and utilization parameters for microbial growth will decrease the net utilization of hydrogen for microbial growth. This effect

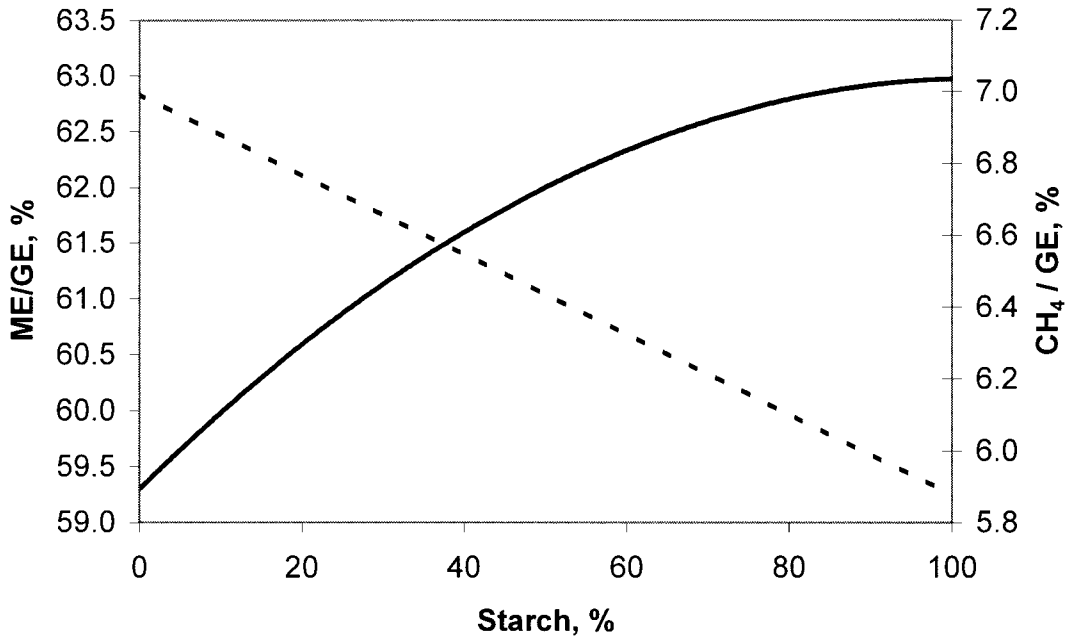

Figure 7. Simulated energy partitioning in lactating dairy cows fed increasing proportions of wheat starch in the concentrate. ME/GE (———); CH_4 % GEI (▪▪▪▪▪).

becomes more pronounced as microbial growth on NPN increases relative to growth on amino acids. On average, 9.14% (SD = 2.64%) of methane was simulated from postruminal fermentation. Hence, this is a significant source of difference between the simulations in this investigation and those performed by Benchaar et al. (1998b). The root MSPE of 15.4% is lower than the 19.9% initially observed by Benchaar et al. (1998b), but it is similar to the 15.4% achieved when correcting dietary inputs for pectins within the rumen model (Benchaar et al., 1998b). Following correction for dietary pectin, Benchaar et al. (1998b) also showed a reduction in the degree of under prediction of mean methane emissions (–0.25 MJ/d vs –1.26 MJ/d).

The increase in model predictions for methane production compared with the simulations of Benchaar et al. (1998b) is smaller than expected. The combined effects of hydrogen flux parameter differences and the incorporation of the large intestinal fermentation module lead to increased methane emissions. However, modification to VFA stoichiometry accounted for a moderation of the overall increase in simulated methane emissions. When the model was rerun using the stoichiometry of Murphy et al. (1982), the mean predicted methane production for the literature experiments increased by 0.2 MJ/d, and the root MSPE also increased (18.9%).

The tendency for underprediction of methane emissions from lactating dairy cows by the model contrasts with the corresponding overprediction involving the full literature data set. Although the literature experiments involved several diets containing either lucerne or grass hay, the main differences between these experiments and those performed at CEDAR were the DMI and animal type. The CEDAR trials involved only lactating dairy cows consuming high DMI. Hence, the evaluation of the model for Trials 1 through 5 represented a substantially different challenge. Reasons for underprediction of methanogenesis by a modified form of the Dijkstra et al. (1992) model have been presented previously (Benchaar et al., 1998b). Variation between observed and predicted results is partly due to errors in model inputs for dietary characteristics. Errors in the analysis of feedstuffs may be significant, but the inherent variations in nutrient composition between samples of the same feedstuff are more important sources of error. This is most evident for the dietary forages. In particular, fresh grass varies significantly in composition, depending on soil type, weather conditions, and time of cutting or grazing. This may explain the low r^2 for the regression between observed and predicted methane production for Trials 3 and 4.

The prediction of VFA molar proportions or, more specifically, differences in observed and predicted ratios of lipogenic to glucogenic VFA will significantly affect model behavior. The revised stoichiometry (Bannink et al., 2000) should have reduced this error in comparison to previous model evaluations (Benchaar et al., 1998b). However, without experimental observations of VFA production for the cows in Trials 1 through 5, it is not possible to directly assess the reliability of the new stoichiometry within this investigation.

Bannink et al. (1997) identified the quantification of rumen pH and the associated input parameters as potential sources of error within the rumen model. The move toward a more dynamic pH as presented within this investigation should help improve model performance. However, the empirical relationship between VFA concentration and rumen pH ignores several important determinants of rumen pH, such as the buffering from saliva and NDF (Argyle, 1989). Because the model performed well with regard to NDF digestibility within this investigation, erroneous pH prediction is unlikely to be a major contributor to the error of prediction for methane production.

The calculation of fractional passage rate for both the rumen and the large intestine could be a source of error within the model because passage rates were not measured in the simulated experiments. However, a lack of knowledge and the ability to quantify the controlling factors behind digesta passage limit the degree of improvement that can be made to this area of modeling rumen function at the present time (Dijkstra and France, 1996). Overprediction of ruminal passage rate would reduce the degree of fermentation per unit of ingested feed and hence reduce simulated methane production. However, nutrient digestibilities were well predicted, and this suggests that erroneous fractional passage rates were an unlikely cause of differences between observed and predicted methane emissions.

Dietary lipid concentration was not reported within the CEDAR calorimetry studies, and model inputs were based on typical ether extract values reported by MAFF (1992). This procedure undoubtedly leads to some error in the simulation of methane emissions due to the contribution of unsaturated fatty acids as a hydrogen sink and the glycerol as an energy source for fermentation. However, the present representation of lipid metabolism in the rumen model is insufficient for the examination of the true effects of manipulating dietary lipid intake and composition on the output of methane. A more detailed description of lipid metabolism in the rumen and large intestine would be required, and in particular, the degree of saturation of feed lipid needs precise characterization for various feedstuffs.

The physical form of individual dietary ingredients can affect the digestion process and methane production (Blaxter, 1989). Processing of cereal grains increases the susceptibility of starch to ruminal degradation (Mills et al., 1999a) while it increases the rate of passage from the rumen (Kennedy and Poppi, 1984; Pond et al., 1984). Diets involving WCW silage can contain substantial quantities of whole-wheat grains, and the starch contained within these grains is less susceptible to rumen degradation than that exposed through grinding or rolling (Mills et al., 1999a). Within the Dijkstra et al. (1992) rumen model, soluble starch is assumed immediately available for microbial growth and fermenta-

tion. However, when whole cereal grains are present, this soluble starch is not released to the microbes. This explains the overprediction of starch degradation for Trial 2. It is likely that the model will overestimate methanogenesis for diets with significant levels of whole cereal grains. Therefore, the degree of underprediction of methanogenesis on Trial 2 was unexpected. Both starch and protein digestibilities are overestimated by the model for Trial 2. The NDF digestibility was in line with experimental observations. Ruminal passage rates may be reduced when high dry matter WCW silage is fed. This could contribute to elevated methane emissions, although it would further elevate the prediction of starch and NDF digestibilities.

Czerkawski (1986) suggests that high methane-to-VFA ratios in the rumen could be the result of acetotrophic methanogenic bacteria. These bacteria utilize acetate for a source of carbon and energy and do not require hydrogen (Huser et al., 1982). The presence of these bacteria within the rumen could explain the underestimation of methane production by the model, although their significance is uncertain. The influence of protozoa on ruminal fermentation and methanogenesis is more established. Methane production was shown to be significantly higher in faunated ($P < 0.001$) than in ciliate-free cattle (Whitelaw et al., 1984). The researchers explained this occurrence in terms of the increased production of glucogenic VFA Whitelaw et al. (1984). Methanogenic bacteria have also been shown to be directly associated with protozoa (Hillman et al., 1988; Finlay et al., 1994; Newbold et al., 1995). Failure of the model to account for the impact of protozoa on ruminal methane production could lead to underestimation of total methane output.

Murray et al. (1999) showed that methane production was raised when sheep were housed in open-circuit respiration chambers compared with polytunnels, with methane recovery being similar for both systems. This research highlights the potential for a link between animal behavior induced by the two systems and methane emissions. If a similar situation exists for dairy cattle, this could explain the model's tendency to underpredict methane emissions.

Czerkawski (1986) estimated that the reduction of carbon dioxide to methane accounted for 48% of hydrogen utilization in the rumen of sheep, with 33% used in VFA production, 12% in microbial synthesis, and 1 to 2% in fatty acid biohydrogenation. The model predictions differed substantially from these estimates. The simulations suggest that the production of methane accounts for almost 80% of hydrogen utilization following fermentation. In reality, there are other hydrogen sinks unaccounted for in the model. Nitrates and sulfates act as hydrogen sinks. However, the main sink unaccounted for within the model structure may be that of oxygen from transfer across the rumen epithelium (Czerkawski, 1986). According to Czerkawski (1986), these other sinks contribute to approximately 5% of total hydrogen use. Therefore, these sinks are unlikely to account for the large differences observed in the prediction of the fate of hydrogen during fermentation. The most notable difference between the estimates of Czerkawski (1986) and the model predictions was the small significance of microbial growth as a hydrogen sink (0.6%). The predominance of microbial growth utilizing amino acids within the model gave rise to a net production of hydrogen for a large part of the microbial population. We conclude either that the model underestimates the total availability of metabolic hydrogen or that previous estimates for microbial growth as a hydrogen sink are inflated.

Implications

This investigation has demonstrated that the mechanistic approach to modeling methanogenesis is reliable for the prediction of methanogenesis in the lactating dairy cow. The ability of the model to simulate methanogenesis for a wide range of dietary inputs allows its application as a tool for determining dietary strategies to reduce environmental impact of dairy systems and to maximize feed energy utilization. This investigation has shown the potential for dietary intervention as a means of substantially reducing methane emissions without adverse affects to dietary energy supply.

Literature Cited

ACSL. 1995. Advanced Continuous Simulation Language. User's Guide/Reference Manual. Mitchell and Gauthier Associates.

AFRC. 1993. Energy and Protein Requirements of Ruminants. Agricultural and Food Research Council. CAB International, Wallingford.

Argyle, J. L. 1989. Modelling rumen water kinetics and pH effects. In: A. B. Robson, and D. P. Poppi (ed.) Proc. 3rd Int. Workshop on Modelling Digestion and Metabolism in Farm Animals. Lincoln University, Canterbury, New Zealand. pp 65–78.

Axelsson, J. 1949. The amount of produced methane energy in the European metabolic experiments with adult cattle. Ann. R. Agric. Coll. Sweden 16:404–419.

Baldwin, R. L. 1995. Modeling Ruminant Digestion and Metabolism. Chapman and Hall, London.

Baldwin, R. L., J. H. M. Thornley, and D. E. Beever.1987. Metabolism of the lactating cow II. Digestive elements of a mechanistic model. J. Dairy Res. 54:107–131.

Bannink, A., H. De Visser, and A. M. Van Vuuren. 1997. Comparison and evaluation of mechanistic rumen models. Br. J. Nutr. 78:563–581.

Bannink, A., J. Kogut, J. Dijkstra, A. M. Van Vuuren, J. France, and S. Tamminga. 2000. Modelling production and portal appearance of volatile fatty acids in dairy cows. In: J. P. McNamara, J. France, and D. E. Beever (ed) Modelling Nutrient Utilisation in Farm Animals. pp 87–102. CABI Publishing, Wallingford.

Belyea, R. L., P. J. Marin, and H. T. Sedgewick. 1985. Utilization of chopped and long alfalfa by dairy heifers. J. Dairy Sci. 68:1297–1301.

Benchaar, C., J. Rivest, C. Pomar, and J. Chiquette. 1998a. Comment on the paper "Prediction of methane production from dairy cows using existing mechanistic models and regression equations". Reply to the letter by K. C. Donovan and R.L. Baldwin. J. Anim. Sci. 76:2752–2753.

Benchaar, C., J. Rivest, C. Pomar, and J. Chiquette. 1998b. Prediction of methane production from dairy cows using existing mechanistic models and regression equations. J. Anim. Sci. 76:617–627.

Bibby, J. and H. Toutenburg. 1977. Prediction and Improved Estimation in Linear Models. John Wiley and Sons, London.

Blaxter, K. L. 1989. Energy Metabolism in Animals and Man. Cambridge University Press, New York.

Bosch, M. W., S. Tamminga, G. Post, C. P. Leffering, J. M. Muylaert. 1992. Influence of stage of maturity of grass silages on digestion processes in dairy cows. 1. Composition, nylon bag degradation rates, fermentation characteristics, digestibility and intake. Livest. Prod. Sci. 32:245–264.

Cammell, S. B., J. D. Sutton, D. E. Beever, D. J. Humphries, and R. H. Phipps. 2000. The effect of crop maturity on the nutritional value of maize silage for lactating dairy cows. Anim. Sci. 71:381–390.

Castillo. A. R.., E. Kebreab, D. E. Beever, and J. France. 1999. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. J. Anim. Feed Sci. 9:1–32.

Coppock, C. E., W. P. Flatt, L. A. Moore, and W. E. Stewart. 1964. Effect of hay to grain ratio on the utilisation of metabolizable energy for milk production by dairy cows. J. Dairy Sci. 47:1330–1338.

Cushnahan, A., C. S. Mayne, and E. F. Unsworth. 1995. Effects of ensilage of grass on performance and nutrient utilisation by dairy cattle. 2. Nutrient metabolism and rumen fermentation. Anim. Sci. 60:347–359.

Czerkawski, J. W. 1986. An Introduction to Rumen Studies. Pergamon Press, Oxford.

Demeyer, D. I. 1991. Quantitative aspects of microbial metabolism in the rumen and hindgut. In: J. P. Jouany (ed) Rumen Microbial Metabolism and Ruminant Digestion. p 217. INRA Editions, Paris.

Dijkstra, J., and J. France. 1996. A comparative evaluation of models of whole rumen function. Ann. Zootech. (Paris) 45:175–192.

Dijkstra, J., H. D. St. C. Neal, D. E. Beever, and J. France. 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. J. Nutr. 122:2239–2256.

Donovan, K. C., and R. L. Baldwin. 1998. Comment on the paper "Prediction of methane production from dairy cows using existing mechanistic models and regression equations". J. Anim. Sci. 76:2751-2752.

Erdman, R. A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71:3246–3266.

Finlay, B. J., G. Esteban, K. J. Clarke, and A. G. Williams. 1994. Some rumen ciliates have endosymbiotic methanogens. FEMS Microbiol. Lett. 117:157–162.

France, J., J. H. M. Thornley, R. L. Baldwin, and K. A. Crist. 1992. On solving stiff equations with reference to simulating ruminant metabolism. J. Theor. Biol. 156:525–539.

France, J., J. H. M. Thornley, R. C. Siddons, and M. S. Dhanoa. 1993. On incorporating diffusion and viscosity concepts into compartmental models for analysing faecal marker excretion patterns in ruminants. Br. J. Nutr. 70:369–378.

Gibbs, M., and D. E. Johnson. 1994. Methane emissions from the digestive processes of livestock. In: International Anthropogenic Methane Emissions Estimates for 1990. EPA publication No. 230-R-93-010.

Harlan, D. W., J. B. Holter, and H. H. Hayes. 1991. Detergent fiber traits to predict productive energy of forages fed free choice to nonlactating cattle. J. Dairy Sci. 74:1337–1353.

Hillman, K., D. Lloyd, and A. G. Williams. 1988. Interactions between the methanogen *Methanosarcina barkeri* and rumen holotrich ciliate protozoa. Lett. Appl. Microbiol. 7:49–53.

Holter, J. B., J. A. Bullis, and H. H. Hayes. 1986. Predicting maternal protein and fat balances of growing and mature dry cows. J. Dairy Sci. 69:2622–2635.

Holter, J. B., M. J. Slotnick, H. H. Hayes, C. K. Bozak, W. E. Urban, and M. L. McGilliard. 1990. Effect of prepartum dietary energy on condition score, postpartum energy, nitrogen partitions, and lactation production responses. J. Dairy Sci. 73:3502–3511.

Holter, J. B., and A. J. Young. 1992. Methane prediction in dry and lactating Holstein cows. J. Dairy Sci. 75:2165–2175.

Huhtanen, P., and A. Vanhatalo. 1997. Ruminal and total plant cell-wall digestibility estimated by a combined in situ method utilizing mathematical models. Br. J. Nutr. 78:583–598.

Huser, B. A., K. Wuhrmann, and A. J. B. Zehnder. 1982. Methanothrix-Soehngenii gen-nov-sp-nov, a new acetotrophic non-hydrogen -oxidizing methane bacterium. Arch. Microbiol. 132:1–9.

Kennedy, P. M. and D. P. Poppi. 1984. 'Critical particle size' in sheep and cattle. In: P. M. Kennedy, (ed.) Techniques in Particle Size Analysis of Feed and Digesta in Ruminants, Canadian Society of Animal Science Occasional Publication no.1. p 170.

Kriss, M. 1930. Quantitative relations of the dry matter of the food consumed, heat production, the gaseous outgo and the insensible loss in body weight in cattle. J. Agric. Res. 40:283.

MAFF. 1992. Feed Composition. UK Tables of Feed Composition and Nutritive Value for Ruminants. 2nd ed. Chalcombe Publications, Canterbury.

Mills, J. A. N., and J. France, J. Dijkstra. 1999a. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 1. Dietary starch characterisation and ruminal starch digestion. J. Anim. Feed Sci. 8:291–340.

Mills, J. A. N., J. France, and J. Dijkstra. 1999b. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 2. Postruminal starch digestion and small intestinal glucose absorption. J. Anim. Feed Sci. 9:451–481.

Moe, P. W., and H. F. Tyrrell. 1972. Net energy value for lactation of high and low protein diets containing corn silage. J. Dairy Sci. 55:318–324.

Moe, P. W., and H. F. Tyrrell. 1973. Physical form and energy value of corn grain. J. Dairy Sci. 56:1298–1304.

Moe, P. W., and H. F. Tyrrell. 1977. Effects of feed intake and physical form on energy value of corn in timothy hay diets for lactating cows. J. Dairy Sci. 60:752–758.

Moe, P. W., and H. F. Tyrrell. 1979a. Effect of endosperm type on incremental energy value of corn grain for dairy cows. J. Dairy Sci. 62:447–454.

Moe, P. W., and H. F. Tyrrell. 1979b. Methane production in dairy cows. J. Dairy Sci. 62:1583–1586.

Moe, P. W., and H. F. Tyrrell, and N. W. Hooven. 1973. Energy balance measurements with corn meal and ground oats for lactating cows. J. Dairy Sci. 56:1149–1153.

Morard, V. 1999. Emissions of biogenic greenhouse gases from agriculture: political background and a European perspective. In: Proc. Conference on Biogenic Greenhouse Gases Caused by Arable and Animal Agriculture. Stuttgart, Germany. pp 1–2.

Murphy, M. R., R. L. Baldwin, and L. J. Koong. 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. J. Anim. Sci. 55:411–421.

Murray, P. J., A. Moss, D. R. Lockyer, and S. C. Jarvis. 1999. A comparison of systems for measuring methane emissions from sheep. J. Agric. Sci. 133:439–444.

Murray, R. M., A. M. Bryant, and R. A. Leng. 1976. Rates of production of methane in the rumen and large intestine of sheep. Br. J. Nutr. 36:1–14.

Murray, R. M., A. M. Bryant, and R. A. Leng. 1978. Methane production in the rumen and lower gut of sheep given lucerne chaff: effect of level of intake. Br. J. Nutr. 39:337–345.

Newbold, C. J., B. Lassalas, and J. P. Jouany. 1995. The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. Lett. Appl. Microbiol. 21:230–234.

Nocek, James E., and Alan L. Grant. 1987. Characterization of in situ nitrogen and fiber digestion and bacterial nitrogen contamination of hay crop forages preserved at different dry matter percentages. J. Anim. Sci. 64:552–564.

Nocek, J. E., and S. Tamminga. 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74:3598–3629.

Palmquist, D. L., M. R. Weisbjerg, and T. Hvelplund. 1993. Ruminal, intestinal, and total tract digestibilities of nutrients in cows fed diets high in fat and undegradable protein. J. Dairy Sci. 76:1353–1364.

Parra, R. 1978. Comparison of foregut and hindgut fermentation in herbivores. In: G. G. Montgomery (ed) The Ecology of Arboreal Folivores. p 205. Smithsonian Institution Press, Washington DC.

Pellikaan, W. F., H. Boer, J. Dijkstra, G. Hof, V. V. A. M. Schreurs, S. Tamminga, and J. Vos. 1999. Passage dynamics of [^{13}C]-enriched feed particles in grass silage fed dairy cows. S. Afr. J. Anim. Sci. 29:274–275.

Pond, K. R., E. A. Tolley, W. C. Ellis, and J. H. Matis. 1984. A method for describing the weight distribution of particles from sieved forage. In: P. M. Kennedy, (ed.). Techniques in Particle Size Analysis of Feed and Digesta in Ruminants. Canadian Society of Animal Science, Occasional Publication no.1. pp 123–133.

Reichl, J. R., and R. L. Baldwin. 1975. Rumen modeling: Rumen input-output balance models. J. Dairy Sci. 58:879–890.

Sutton, J. D., R. H. Phipps, S. B. Cammell, and D. J. Humphries. 2001. Attempts to improve the utilization of urea-treated whole-crop wheat by lactating dairy cows. Anim. Sci. 72(In press).

Sutton, J. D., I. C. Hart, W. H. Broster, R. J. Elliot, and E. Schuller. 1986. Feeding frequency for lactating dairy cows: effects on rumen fermentation and blood metabolites and hormones. Brit. J. Nutr. 56:181–192.

Tamminga, S., and A. M. Van Vuuren. 1988. Formation and utililisation of end products of lignocellulose degradation in ruminants. Anim. Feed Sci. Tech. 21:141–159.

Tamminga, S., A. M. Van Vuuren, C. J. Van der Koelen, R. S. Ketelaar, and P. van der Togt. 1990. Ruminal behaviour of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. Neth. J. Agric. Sci. 38:513–526.

Tyrrell, H. F., and P. W. Moe. 1972. Net energy value for lactation of a high and low concentrate ration containing corn silage. J. Dairy Sci. 55:1106–1112.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca.

Van Vuuren, A. M., S. Tamminga, and R. S. Ketelaar. 1990. Ruminal availability of nitrogen and carbohydrates from fresh and preserved herbage in dairy cows. Neth. J. Agric. Sci. 38:499–512.

Vanhatalo, A., and E. Ketoja. 1995. The role of the large intestine in post-ruminal digestion of feeds as measured by the mobile-bag method in cattle. Br. J. Nutr. 73:491–505.

Whitelaw, F. G., J. M. Eadie, L. A. Bruce, and W. J. Shand. 1984. Methane formation in faunated and ciliate-free cattle and its relationship with rumen volatile fatty acid proportions. Br. J. Nutr. 52:261–275.

Wilkerson, V. A., D. P. Casper, and D. R. Mertens. 1995. The prediction of methane production of Holstein cows by several equations. J. Dairy Sci. 78:2402–2414.