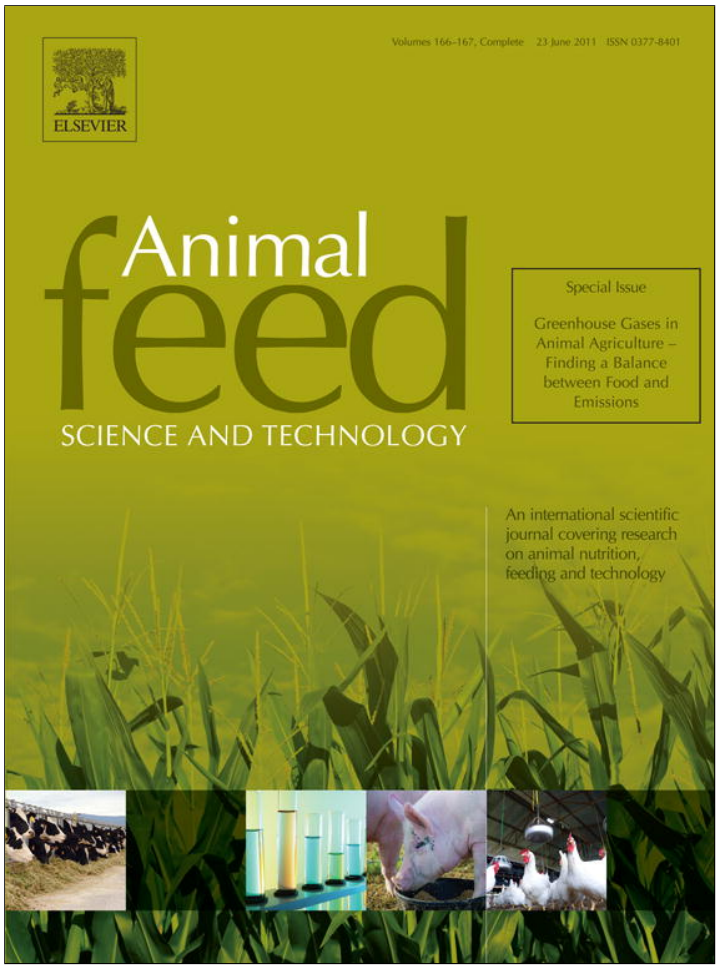

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A model of enteric fermentation in dairy cows to estimate methane emission for the Dutch National Inventory Report using the IPCC Tier 3 approach

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ABSTRACT

The protocol for the National Inventory of agricultural greenhouse gas emissions in The Netherlands includes a dynamic and mechanistic model of animal digestion and fermentation as an Intergovernmental Panel on Climate Change (IPCC) Tier 3 approach to estimate enteric CH_4 emission by dairy cows. The model differs from an IPCC Tier 2 approach in that it predicts hydrogen sources (*i.e.*, production of acetate and butyrate, microbial growth on amino acids as an N source) and sinks (*i.e.*, production of propionate and the remainder of the volatile fatty acids (VFA), microbial growth on ammonia as an N source, saturation of unsaturated long chain fatty acids) in the rumen and large intestine, and elimination of excess hydrogen by methanogenesis. As a result, the model predicts CH_4 emission by considering various dietary characteristics, including the types of carbohydrate, protein, fat, intrinsic degradation characteristics of feeds, as well as ruminal fractional passage rates, fluid volume and acidity, instead of assuming a fixed CH_4 energy conversion factor in the Tier 2 approach. Annual statistics of diet and performance of the average dairy cow in The Netherlands from 1990 until 2008 indicate that dry matter intake and yield of fat and crude protein corrected milk (FPCM) per cow/year increased by 20 and 34% respectively. Based on annual data for diet and FPCM, the model predicted an increase in enteric CH_4 emission from 111 (1990) to 128 (2008) kg/cow/year. As a result, CH_4 emission per kg FPCM milk decreased by 13%. The predicted fraction of gross energy intake lost as CH_4 energy gradually declined and was close to 0.06, which is the IPCC (1997) Tier 2 default value of 0.06 for dairy cows, but ~10% lower than the IPCC (2006) updated value of 0.065. The 15% uncertainty value for predicted CH_4 emissions for a reference diet was lower than the 20% assumed under Tier 2. Our analysis indicated that uncertainty of model predictions of CH_4 emission is determined mostly by errors in feed intake estimation, in the representation of the stoichiometry of production of VFA from fermented substrate, and in the acidity of rumen contents. Further uncertainty of predicted CH_4 emission was due to errors in estimation of dietary composition

Abbreviations: CP, crude protein; *D*, potentially degradable fraction in the rumen; DM, dry matter; FPCM, fat and protein corrected milk; GE, gross energy; GHG, greenhouse gas; IPCC, Intergovernmental Panel on Climate Change; MCF, CH_4 energy conversion factor; MEF, CH_4 emission factor; NE_l, net energy for lactation; ST, starch; SU, sugars and soluble carbohydrates; *U*, undegradable fraction in the rumen; VEM, unit used to express net energy for lactation in the Dutch NE_L system for dairy cows; VFA, volatile fatty acids; *W*, washable fraction of feeds in the rumen.

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of ingredients and in chemical compositions of dietary components. Results demonstrate that prediction of CH_4 should not solely focus on representing effects of nutrition on overall digestion and apparent feed utilization by cows, but that additional attention is needed to address effects of nutrition on intra-ruminal fermentation conditions, and their effects on formation of VFA and the rumen hydrogen balance.

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1. Introduction

Emission of CH_4 by cattle as a result of microbial fermentation in the rumen and large intestine contributes to worldwide greenhouse gas (GHG) emissions (Steinfeld et al., 2006). In The Netherlands, dairy cows emit by far the largest part of CH_4 from agriculture. When expressed in CO_2-eqv, the contribution of total CH_4 from dairy operations is of the same order of magnitude as the contribution of total N_2O from Dutch agriculture. For both gases, agriculture contributes slightly more than 50% of total national emissions (Van der Maas et al., 2010). Because of the marked contribution of dairy cows to national CH_4 emissions, and because CH_4 emission factors vary with feed intake and diet composition, emphasis has been placed on obtaining accurate estimates of enteric CH_4 emission in dairy cows by introduction of an Intergovernmental Panel on Climate Change (IPCC) Tier 3 approach in the Dutch protocol of the National Inventory on GHG emissions (Van der Maas et al., 2010).

According to IPCC guidelines for a Tier 2 approach (IPCC, 1997), enteric CH_4 emission in high yielding cows (MEF; CH_4 emission factor in kg CH_4/cow/yr) is estimated from emitted CH_4 energy (MJ/yr), with the latter estimated from gross energy (GE) intake. Intake of GE is calculated based on net energy for lactation (NE_l) requirements for maintenance and production, and on NE_l and GE contents of diets fed, using standard national feeding systems and for which data are available from monitoring studies and national statistics. A default value for the proportion of GE intake as CH_4 energy emission (*i.e.*, CH_4 conversion factor, MCF) of 0.060 is used in the IPCC (1997) Tier 2 approach to estimate CH_4 emissions from GE intake. The MCF value was increased to 0.065 by IPCC in 2006. However such default, fixed, estimates and standard calculations may not address the variation encountered in commercial production due to cow type (*i.e.*, feed intake, cow productivity), diet composition and dietary characteristics. Ellis et al. (2010) demonstrated that enteric CH_4 prediction accuracy using a fixed MCF is low, and application of fixed MCF in whole farm models may introduce substantial error into inventories of GHG emissions. Such error may lead to incorrect mitigation recommendations. To obtain more accurate estimates of enteric CH_4 emissions, the IPCC recommends a Tier 3 approach which makes use of local data from monitoring, experiments and validated calculation methods.

In The Netherlands, a country specific Tier 3 approach was developed to estimate enteric CH_4 emission by dairy cows and has been in use since 2005. This Tier 3 method considers characteristics of microbial fermentation processes in the gastrointestinal tract of cows, and quantifies consequences of feed intake and dietary characteristics on MCF. We outline the various aspects of this Tier 3 approach used in the Dutch protocol for the National Inventory of GHG emission. Results are presented for historic data from 1990 to 2008, and an evaluation of uncertainty of CH_4 predictions as a result of error in model inputs, and in some important internal model parameters and calculation rules in the model, are demonstrated.

2. Methodology

2.1. Tier 3 approach for estimating enteric methane in dairy cows

2.1.1. Model description

A schematic representation of the modelling approach adopted by Dijkstra et al. (1992), Mills et al. (2001) and Bannink et al. (2008, 2010), and currently implemented as a Tier 3 approach to estimate enteric CH_4 emission in dairy cows in the Inventory Report on GHG emissions in The Netherlands is Fig. 1. The model represents dynamics in time and interactions among pool sizes of substrates, microbial matter and other fermentation end products which affect enteric fermentation conditions. These features make the modelling approach fully mechanistic and dynamic by representing effects of nutrition on enteric fermentation and methanogenesis.

The model describes mechanisms underlying microbial degradation and subsequent utilization of feed particles and solutes, microbial growth and formation of volatile fatty acids (VFA) and H_2 as rumen fermentation end products. The model describes flows to and from pools of substrates, microorganisms and fermentation end products in the rumen as well as the large intestine. Production and utilization rates with respect to pools of substrate, microbial matter and fermentation end products are usually concentration dependent and described by nonlinear relationships based on enzyme kinetics. The modelling efforts of Baldwin et al. (1987), based on early efforts of Baldwin et al. (1970) and France et al. (1982), adopted a comparable approach to the approach used in our study, as discussed by Bannink and De Visser (1997a) and Bannink

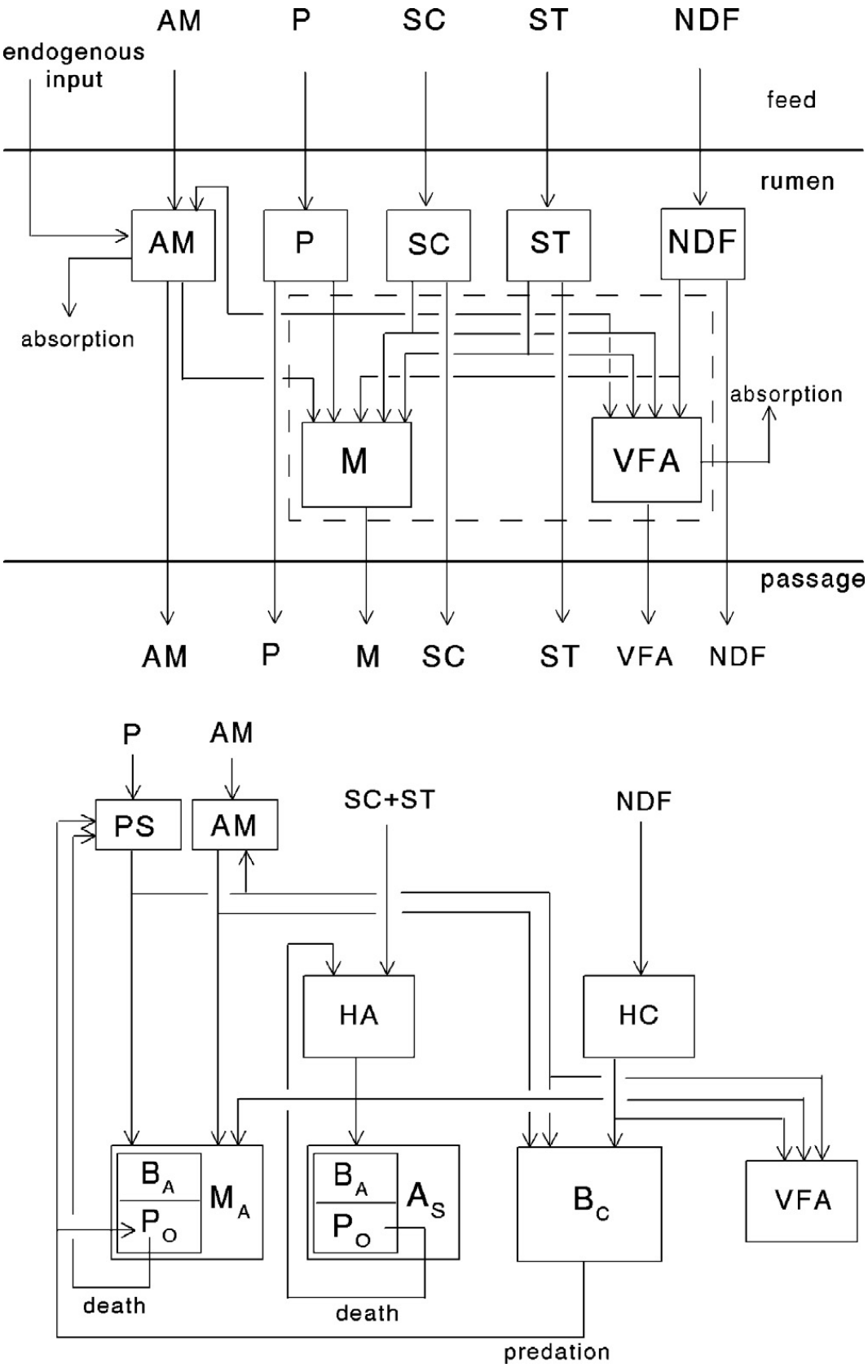

Fig. 1. (A) Basic scheme of interactions between substrate and microbial pools and consequences for end products of fermentation in the dynamic mechanistic model currently used as the Dutch Tier 3 approach for enteric CH_4 emission in dairy cattle (Dijkstra et al., 1992). Depicted are flows to and from the following rumen pools: AM, ammonia; P, protein; SC, soluble carbohydrates; ST, starch; NDF, aNDFom; M, microbial mass; VFA, volatile fatty acids. (B) A more detailed diagram of the mechanism of microbial activity depicted in the dotted square in (A) with flows to and from the following pools: A_S, storage polysaccharides of amylolytic microbes; B_A, amylolytic bacteria; B_C, cellulolytic bacteria; HA, soluble rumen hexose originating from SC and ST; HC, soluble rumen hexose originating from NDF; M_A, amylolytic microbes; P_O, protozoa. Other abbreviations as described in (A), M in (A) corresponding to B_A, B_C

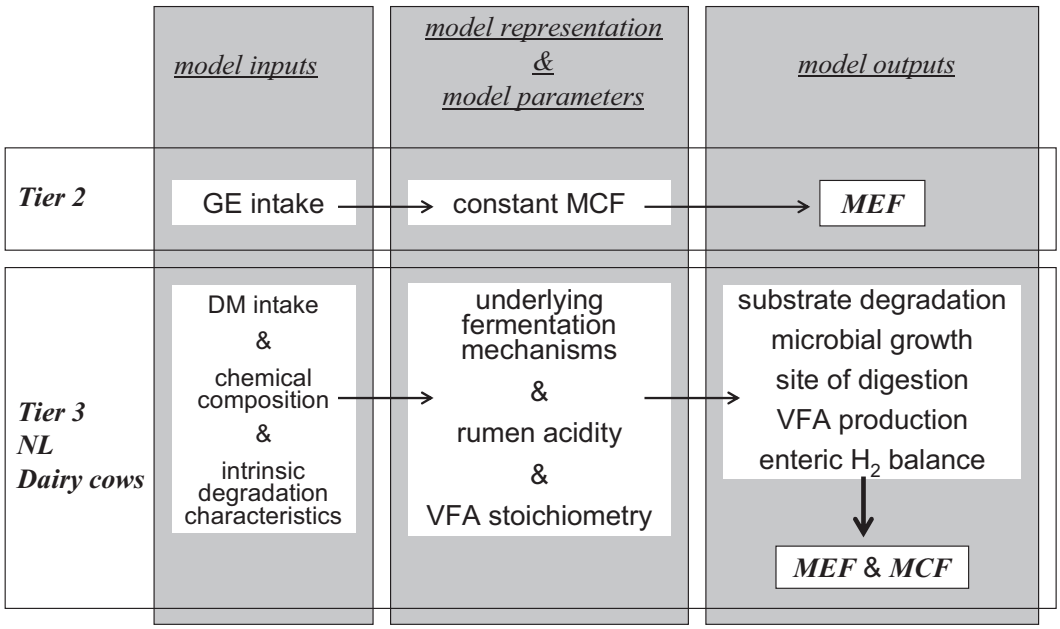

Fig. 2. Schematic representation of the distinction between calculation rules for enteric CH_4 emission in dairy cows (indicated by grey frames) according to the Tier 2 approach following IPCC guidelines (IPCC, 1997; GE, gross energy) and the current Dutch Tier 3 approach to estimate MEF (kg CH_4/cow/yr) and MCF (CH_4 energy as a fraction of GE intake with feed). H_2, hydrogen gas; VFA, volatile fatty acids.

et al. (1997c), including a detailed model comparison and evaluation. The approach differs from a Tier 2 approach (Fig. 2), because it represents enteric fermentation processes and predicts CH_4 as an outcome without *a priori* assumptions on some parameters related to CH_4, such as a MCF in Tier 2.

The mathematical notation of the model of rumen digestion published by Dijkstra et al. (1992), includes all algorithms and parameter values. A diagram of the rumen processes in this model is Fig. 1, including interactions between feed substrates and microorganisms, and production of end products of fermentation (*i.e.*, VFA, ammonia, microbial matter). The model describes interactions between 3 groups of micro-organisms (*i.e.*, amylolytic bacteria, cellulolytic bacteria, protozoa) and 4 substrate pools (*i.e.*, ammonia, soluble protein, amylolytic hexose, cellulolytic hexose). Inputs to these substrate pools are either direct from ingested feed (*i.e.*, ammonia, rumen washable crude protein (CP), sugars, rumen washable starch) or from feed substrates degraded by microorganisms (*i.e.*, rumen degradable starch, rumen degradable aNDFom, rumen degradable CP). The rumen degradation rate of a specific substrate depends on its pool size, on the pool size of utilizing microorganisms, rumen pH, and the intrinsic fractional degradation characteristics of the substrate. The latter are derived from rumen *in situ* incubation of feeds in the rumen. Crude fat is represented as a pool, but no interaction was assumed between microbial activity and fat in the rumen. Bacterial and protozoal growth is represented in a mechanistic manner by making a distinction between substrate utilized to generate metabolic energy and substrate utilized for incorporation with resultant biosynthesis of microbial matter in the rumen. Additionally, utilization of substrate for growth and non-growth (*i.e.*, maintenance) is represented. The type of VFA formed in rumen fermentation depends on the type of substrate fermented (*i.e.*, soluble carbohydrates and sugars (SU), starch (ST), hemicellulose, cellulose, protein). Based on an empirical study on the relationships between rumen substrate digestion and VFA molar proportions in rumen fluid (Murphy et al., 1982), a distinction was made between VFA produced with forage rich diets, concentrate rich diets and intermediate diets.

Mills et al. (2001) added a representation of intestinal digestion and fermentation, as well as a representation of H_2 and CH_4 production to the model of Dijkstra et al. (1992). Also, the stoichiometry of VFA production from fermented substrate developed by Murphy et al. (1982), used in the original model version of Dijkstra et al. (1992), was replaced by the VFA stoichiometry developed by Bannink et al. (2000) and later published by Bannink et al. (2006). Calculation of net H_2 production follows the stoichiometry of VFA production, microbial growth and biohydrogenation of unsaturated fatty acids. There is net production of H_2 with production of acetate and butyrate and, with microbial growth using amino acids as a N source, there is net utilization of H_2 with production of propionate and the remainder of VFA, with microbial growth using ammonia as a N source, and in biohydrogenation of unsaturated long chain fatty acids. Production of CH_4 was calculated assuming conversion of net H_2 excess (*i.e.*, net H_2 production minus net H_2 utilization) into CH_4 by methanogens (Fig. 3). Thus, H_2 and CH_4 were represented as zero pools, implying that the model did not represent the dynamics of the pool of active methanogens. Stoichiometric coefficients for VFA production were derived from Bannink et al. (2006) who gave a description of the coefficient values derived for forage and concentrate rich diets, and included an evaluation of the applicability and

and P_O (B_A and P_O including A_S) in (B), and passage and absorption flows are not represented. In principle, (A) and (B) hold for fermentation in the rumen as well as the large intestine, except for the absence in the large intestine of P_O (death and predation of bacteria by protozoa) and endogenous inputs. Both figures are reproduced with permission from Bannink et al. (1997d).

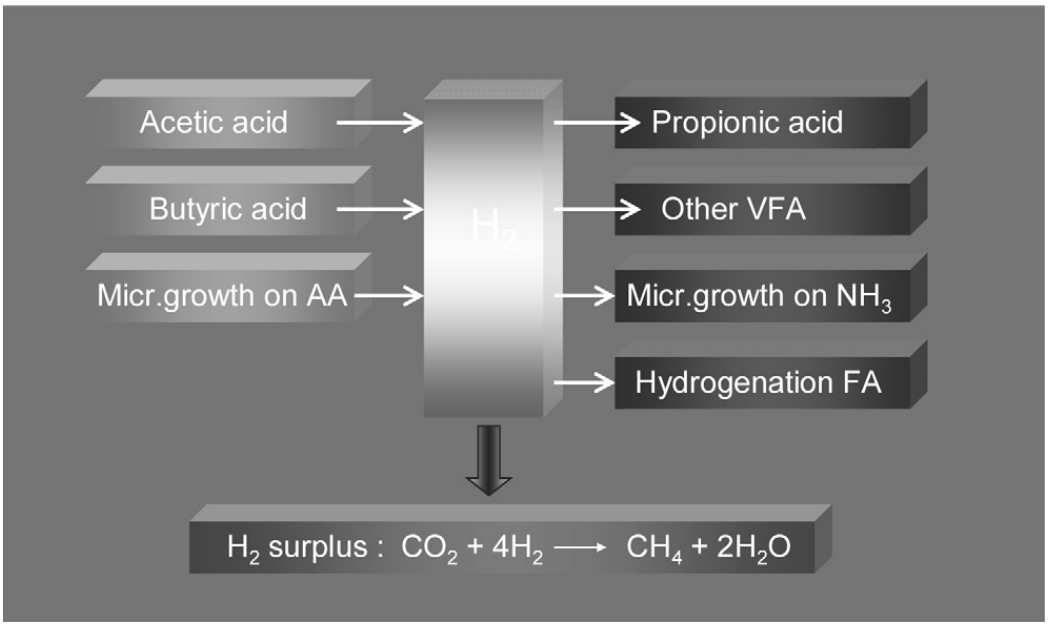

Fig. 3. Schematic representation of the main driving variables for H_2 excess and methanogenesis according to the representation of Mills et al. (2001): the amount and pattern of volatile fatty acids (VFA) produced (Bannink et al., 2008, 2010). Also some factors of minor importance are indicated, being microbial growth on difference N sources (*i.e.*, ammonia or amino acids, AA) and hydrogenation of unsaturated fatty acids (FA).

accuracy of the nonlinear regression procedures used. The reason to replace the VFA stoichiometry of Murphy et al. (1982) was that model evaluations indicated high inaccuracy of predicted VFA molar proportions in rumen fluid (Neal et al., 1992; Bannink et al., 1997b,c). In contrast to Murphy et al. (1982), Bannink et al. (2006) used *in vivo* data on rumen fermentation characteristics and duodenal flows of multiple fistulated lactating dairy cows only. For further details, explanation, and the full mathematical notation of methanogenesis in the rumen and large intestine, see the study of Mills et al. (2001).

More recently, the representation of VFA stoichiometry has been updated by Bannink et al. (2008, 2010). An analysis of literature data was completed, similar to that by Bannink et al. (2006) but, in addition, effects of rumen fluid volume, fractional fluid passage rate, rumen pH and a nonlinear equation for fractional rate of VFA absorption were accounted for in the regression model. The factors involved in rate of VFA absorption have been described in detail by Dijkstra et al. (1993). Furthermore, the constant VFA coefficients for conversion of soluble carbohydrates and starch to individual VFA were replaced by logistic equations that described pH dependency of the conversion of fermented substrate into individual VFA (Bannink et al., 2008, 2010). All factors were considered with regression of the nonlinear model against *in vivo* observations of rumen digestion. In this respect, Bannink et al. (2008, 2010) took a different approach from that adopted by Argyle and Baldwin (1988) and Pitt et al. (1996) based on *in vitro* data. Incorporation of the VFA stoichiometry of Bannink et al. (2008, 2010) into the model of Mills et al. (2001) has been in use as a Tier 3 approach to estimate enteric CH_4 emissions from dairy cows for the Dutch National Inventory on GHG emissions since 2005. The Tier 3 applies an identical representation of rumen microbial activity and methanogenesis as described by Dijkstra et al. (1992) and by Mills et al. (2001), respectively.

2.1.2. Model inputs

The model requires inputs on feed intake (kg dry matter (DM)/d), chemical composition of dietary DM and *in situ* degradation characteristics of aNDFom, ST and CP (Table 1). Missing data on chemical composition, in particular concentrates and wet by-product feeds, were derived from feed tables (CVB, 2007). Chemical fractions identified were aNDFom, ST, SU, total CP, non-ammonia CP, crude fat, ash and, in the case of silages, organic acids. The remainder of DM not explained by these chemical fractions was added (50/50 basis) to aNDFom and SU in case of starch poor feeds (*i.e.*, grass products, beet pulp, wet brewers grains), and added (50/50 basis) to aNDFom and starch in case of starch rich feed (*i.e.*, maize silage, potato by-products, concentrates).

In situ rumen degradation characteristics required as a model input are the rumen washable fraction (W), the potentially degradable fraction in the rumen (D), the rumen undegradable fraction (U) and an estimate of the fractional rate constant of degradation of D in the rumen for ST, aNDFom and CP (Table 1). Estimates of these degradation parameters were derived from databases on degradation of feeds which have been incubated in the rumen in nylon bags, or general estimates were used.

The Dijkstra et al. (1992) model requires some additional parameters as input, such as rumen fluid volume, rumen fractional rates of fluid outflow and outflow of particulate matter, rumen average and minimum pH, and the time period with pH <6.3 (Table 1). Adding a model for microbial fermentation and methanogenesis in the large intestine required another set of parameter inputs specific to the large intestine. Intake of DM is the main determinant of the value of these parameters and, for this reason, Mills et al. (2001) derived empirical equations to estimate these parameter inputs from DM

Table 1
Summary of model inputs required by the Tier 3 approach to estimate CH_4 emission for dairy cattle in the National Inventory of GHG emission in The Netherlands (Van der Maas et al., 2010).

Feed intake
DM intake (kg DM/d)
Chemical composition of the dietary DM
Sugars/soluble carbohydrates (g/kg DM)
Starch (g/kg DM)
aNDFom (g/kg DM)
Crude protein (g/kg DM)
Ammonia N (g NH_3-N/g total N)
Fat (g/kg DM)
Ash (g/kg DM)
Fermentation products (g/kg DM)
Input parameters: degradation characteristics
Fraction of rumen washable starch (g/g starch)
Fraction of potentially rumen degradable starch (g/g starch)
Fractional degradation rate of potentially rumen degradable starch (per d)
Fraction of cellulose in aNDFom (g cellulose/g aNDFom)
Fraction of potentially rumen degradable aNDFom (g/g aNDFom)
Fractional degradation rate of potentially rumen degradable aNDFom (per d)
Fraction of rumen undegradable aNDFom (g/g aNDFom)
Fraction of rumen washable crude protein (g/g crude protein)
Fraction of potentially rumen degradable crude protein (g/g crude protein)
Fractional degradation rate of potentially rumen degradable crude protein (per d)
Fraction of rumen undegradable crude protein (g/g crude protein)
Input parameters: fermentation conditions (in rumen and large intestine)[a]
Fractional rumen passage rate of particulate matter (per d)
Fractional rumen passage rate of fluid (per d)
Volume of rumen fluid (L)
Average rumen pH
Minimum rumen pH
Time period rumen pH <6.3 (h)

[a] In the Tier 3 approach, these input parameters are not required as an input but are estimated by empirical equations in the model. See Section 2.1.1 for further explanation.

intake (*i.e.*, rumen fractional passage rates, volume) and predicted rumen pool sizes of VFA (pH parameters) as explanatory variables. These empirical equations were also applied in the Tier 3 model.

2.1.3. Dairy cow data

Model predictions of MEF and MCF are based on national statistics of numbers of dairy cows, annual milk yields per cow, diet composition and DM intake (CBS, 2009). Diet composition and DM intake are estimated from calculated requirements of net energy for lactation (NE_l) considering requirements for maintenance, milk production, growth and pregnancy. The Dutch NE_l system for dairy cows was used with NE_l expressed in units of VEM and 1 VEM equal to 6.9 kJ of NE_l (Van Es, 1978). Some additional energy costs are included which increase total NE_l requirements under practical conditions (Tamminga et al., 2004). Furthermore, maintenance NE_l requirements for high yielding cows are higher than those estimated for low yielding dairy cows in the 1960s and 1970s (Kebreab et al., 2003), which are the basis for several NE_l systems, including the VEM system. Thus, a 10% higher NE_l requirement compared to the basal NE_l requirement calculated for maintenance and milk production (Van Es, 1978) was adopted. A 10% higher NE_l requirement closely matches NE_l requirements monitored on dairy farms in practice and in experiments (Tamminga et al., 2004). National statistics are available on the area used for forage production including separation between the areas used for maize silage production, grass silage production and grazing, which allows estimation of the proportion of each of these forages in the average diet. Further, statistics are available for quantities of standard and protein rich concentrates and wet by-product feeds purchased by dairy farmers.

Details on chemical composition and feeding value of forages are derived from results of a commercial laboratory (*i.e.*, BLGG, Oosterbeek, The Netherlands) for soil and crop analysis. This laboratory completes most forage nutrient analyses in The Netherlands. Dairy farmers in The Netherlands have their grass and maize silages, used for almost all of the milk produced, sampled and analysed by Near Infrared Spectroscopy and, to lesser extent, their grass herbages as well. This analysis provides information on the chemical composition of the silages, in combination with feeding values in NE_l units (Van Es, 1978) and according to the recently updated protein evaluation system (Van Duinkerken et al., 2011). Because most forage produced on dairy farms is covered by this analysis, these data give a good indication of seasonal and yearly variation in composition and quality of the most common forages fed to dairy cows (Tamminga et al., 2004).

General characteristics of concentrates and wet by-product feeds are collected from feed manufacturers and the animal feed industry. Milk production on dairy farms is closely monitored and its quality is tested regularly. Milk data are gathered by the Dutch Dairy Board (Productschap Zuivel, Zoetermeer, The Netherlands) and give an estimate of the production and quality of milk within each region of The Netherlands.

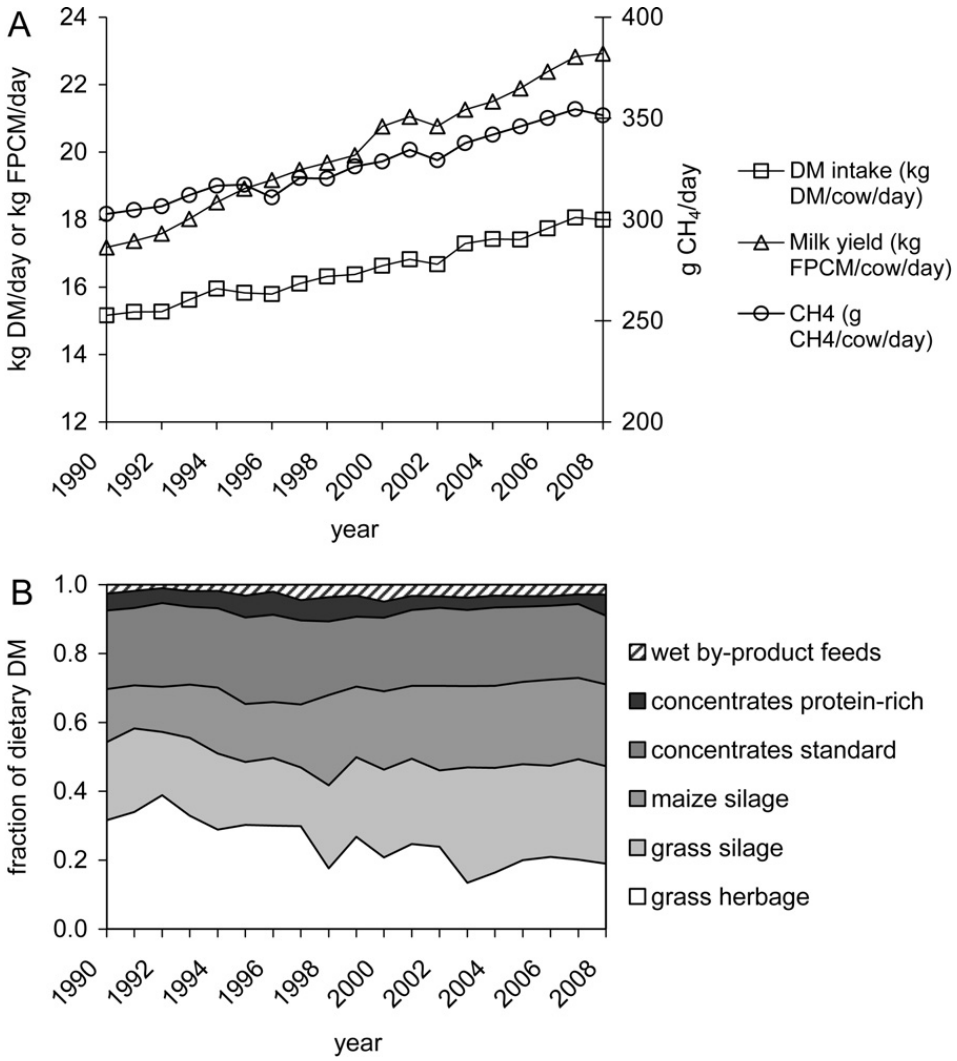

Fig. 4. Evolution of the nutrition and performance of the average dairy cow in The Netherlands from 1990 until 2008 (www.cbs.nl). (A) Changes in DM intake (kg DM/cow/d), production of fat and protein corrected milk (FPCM; kg/cow/d), and CH_4 emission (g CH_4/cow/d). (B) Change in dietary composition. The DM of wet by-product feeds was assumed to be 25% brewers grains, 25% by-products from potato processing, and 50% pressed beet pulp (DM basis) from 1990 until 2003; 25%, 40% and 35% from 2004 until 2006; 31%, 42% and 27% for 2007; 32%, 43% and 25% for 2008.

Based on this information, the average diet of a dairy cow is determined for the Northwest and the Southeast of The Netherlands. Based on milk production realized, the NE_l requirement is calculated and the part not covered by NE_l intake with grass silage, maize silage, concentrates and wet by-product feeds is considered to have come from fresh herbage. From the two average diets per region, and the associated number of dairy cows, a weighed national average diet of dairy cows is calculated. The method is similar to the approach described by Tamminga et al. (2004) to estimate national N and P excretion by dairy cows. The Central Bureau for Statistics (CBS, 2009), evaluated by the Working group Unifying Manure and excretion data (WUM), is responsible for collection of the dairy cow data described above, as well as for calculation of the diet composition of the average dairy cow in The Netherlands.

2.2. Diet and cow statistics

Development of DM intake and milk yield of the average Dutch dairy cow is in Fig. 4A. Total DM intake increased by 20% from 5.5 to 6.5 tonnes of feed DM/cow/yr from 1990 to 2008, an annual increase of 0.164 kg feed DM cow/d (R^2 = 0.98). Annual yield of fat and crude protein corrected milk (FPCM) increased by 34% from 6.3 to 8.4 tonnes of FPCM/cow/yr, an annual increase of 0.325 kg FPCM/cow/d (R^2 = 0.99). Hence, the amount of FPCM produced/kg of ingested feed DM increased by 12%, from 1.13 in 1990 to 1.27 kg FPCM/kg feed DM in 2008.

The composition of dietary DM of the average national diet of dairy cows from 1990 till 2008 (CBS, 2009) is in Fig. 4B. National statistics on intake of these components in kg DM/d by the average adult Dutch dairy cow includes the non-lactating period, but exclude the period until first calving. From 1990 onwards, the proportion of grass herbage in the diet decreased, and that of grass and maize silage increased. The proportion of concentrates in dietary DM decreased slightly from 27% in 1990 to 24% in 2008. Dietary proportions of commercial concentrates and wet by-product feeds in dairy rations were based

Table 2
The chemical composition (g/kg DM) and the NE_L feeding value (units of VEM/kg DM) for grass herbage from 1990 until 2008 (www.cbs.nl).

Year	VEM[a]	Ash	Crude protein	Crude fat	aNDFom	Sugars	Fermentation products
1990	NA[b]	106	268	40	479	97	0
1991	995	110	263	40	479	97	0
1992	1030	110	252	40	479	97	0
1993	991	107	257	40	479	97	0
1994	1003	107	259	40	479	97	0
1995	1008	104	259	40	479	97	0
1996	1033	107	273	40	479	97	0
1997	NA	108	253	40	479	86	0
1998	1020	107	255	40	479	92	0
1999	1012	105	230	40	524	105	0
2000	1005	108	232	40	442	95	0
2001	994	107	229	40	479	93	0
2002	990	105	227	40	508	92	0
2003	977	107	227	40	432	108	0
2004	970	108	206	40	475[c]	117	0
2005	975	107	207	40	475	120	0
2006	957	104	200	40	475	109	0
2007	930	104	191	40	475	113	0
2008	932	105	202	40	511[d]	102	0

[a] VEM is the unit of net energy for lactation used in the energy evaluation system for ruminants in The Netherlands (Van Es, 1978). A unit of VEM corresponds to 6.9 kJ of net energy for lactation.
[b] Not available. Estimates made based on other years.
[c] Value based on aNDFom analysis in 2003.
[d] Only since 2008 is an estimate for aNDFom content available from CBS data. Apart from data in footnote (c), other data were estimates of Smink et al. (2005).

on estimates of amounts purchased by dairy farmers, including a distinction between protein rich and standard concentrates, and estimates of the proportion of individual wet by-product feeds purchased. Wet by-products were distinguished into brewers grains, potato processing and pressed beet pulp.

2.2.1. Chemical composition of dietary components

Tables 2 and 3 show the change in chemical composition of forages over the past two decades. Less data were available with respect to the composition of maize silage and commercially available concentrates than that of grass herbage and grass silage (Table 4). The CP content of grass herbage and silage gradually decreased by 25% and 12%, respectively, partly as a result of legislation in The Netherlands. This decline in CP content was accompanied by an increase of aNDFom in grass silage. The SU content does not seem to have increased in grass silage, whereas it increased in grass herbage. Despite lower fertilization rates of grasslands, analysed NE_l values of grass silage did not appear to decline (Table 3). Since 2003, all details were analysed for maize silage. For commercial concentrates, only data on CP content were obtained from feed manufacturers. Based on feeding tables and general ingredients in commercially available compound feeds, an estimate was

Table 3
The chemical composition (g/kg DM) and the NE_l feeding value (units of VEM/kg DM) for grass silage from 1990 until 2008 (www.cbs.nl).

Year	VEM[a]	Ash	Crude protein	Fraction ammonia in CP (g/kg CP)	Crude fat	aNDFom	Sugars	Fermentation products
1990	868	119	189	60	40	493	78	50
1991	838	125	177	60	40	493	78	50
1992	857	121	184	60	40	493	78	50
1993	861	118	179	60	40	493	78	50
1994	863	118	179	60	40	493	78	50
1995	839	115	179	60	40	493	90	50
1996	874	134	209	60	40	493	58	50
1997	845	125	183	60	40	493	64	50
1998	868	123	176	60	40	479	63	50
1999	879	111	179	60	40	463	101	50
2000	877	120	178	60	40	493	65	50
2001	893	106	174	60	40	486	108	50
2002	863	116	167	60	40	510	74	50
2003	847	112	159	60	40	530	82	50
2004	896	111	173	94	40	489	78	50
2005	897	109	160	88	40	481	99	50
2006	891	101	168	80	33	504	98	50
2007	876	106	161	84	37	511	83	50
2008	888	107	161	80	40	497	88	50

[a] VEM is the unit of net energy for lactation used in the energy evaluation system for ruminants in The Netherlands (Van Es, 1978). A unit of VEM corresponds to 6.9 kJ of net energy for lactation.

Table 4
Chemical composition (g/kg DM) and NE_l (units of VEM/kg DM) of maize silage, standard concentrates and protein rich concentrates from 1990 until 2008 (www.cbs.nl).

	VEM[a]	Ash	Crude protein	Ammonia in CP (g/kg CP)	Crude fat	aNDFom	Sugars	Starch	Fermentation products
Maize silage									
1990–2003	NA	42	74	60	30	433	15	371	35
2004	960	41	71	72	30	412	13	348	35
2005	940	41	71	81	30	432	13	332	35
2006	977	40	79	102	31	382	14	356	35
2007	963	38	70	77	37	393	13	342	35
2008	962	39	73	53	36	388	13	342	35
Standard concentrates									
1990–2003	1080	100	180	0	50	320	100	250	0
2004	1080	100	178	0	50	320	100	250	0
2005	1080	100	179	0	50	320	100	250	0
2006	1080	100	179	0	50	320	100	250	0
2007	1080	100	174	0	50	320	100	250	0
2008	1080	100	166	0	50	320	100	250	0
Protein rich concentrates									
1990–2003	1080	100	330	0	50	270	70	180	0
2004	1080	100	244	0	50	270	70	180	0
2005	1080	100	244	0	50	270	70	180	0
2006	1080	100	241	0	50	270	70	180	0
2007	1080	100	239	0	50	270	70	180	0
2008	1080	100	245	0	50	270	70	180	0

[a] VEM is the unit of net energy for lactation used in the energy evaluation system for ruminants in The Netherlands (Van Es, 1978). A unit of VEM corresponds to 6.9 kJ of net energy for lactation. The NA for maize silage indicates no values were available and average values were used to estimate the diet of the average Dutch dairy cow (www.cbs.nl). The VEM value of maize silage is in itself not a required input for the Tier 3 approach, but the DM intake and chemical composition of DM are.

made of the chemical composition of the remainder of the concentrate DM, which was kept constant (Table 4). The chemical composition of wet by-product feeds was estimated from feeding table values (CVB, 2007) and reported proportions of different types of wet by-products included in dairy rations (Fig. 4B).

2.2.2. Intrinsic degradation characteristics

For all dietary components, the intrinsic degradation characteristics for potentially degradable ST, aNDFom and CP were estimated because these data are not available from standard feed analysis completed in practice. Therefore, general estimates were derived from previous experiments with rumen *in situ* incubations of similar forages and feed ingredients, and from databases available in our laboratories. Table 5 indicates the assumptions for forages and standard and protein rich concentrates. The NE_l value and the chemical composition of maize silage and concentrates did not change much over time (Table 4), and so their degradation characteristics were not changed. Despite the gradual decline in CP content in grass silage, there was no distinct effect on the NE_l. Hence, up to the monitoring year 2008, there was no reason to change the degradation characteristics of grass silage. For grass herbage (Table 2) the decline in CP content was more evident, which may have affected NE_l value (Tamminga et al., 2004). Effects on SU and aNDFom contents were not pronounced and rumen degradation characteristics of grass herbage were not varied.

Table 5
Intrinsic degradation characteristics[a] of potentially rumen degradable starch (ST), cell wall carbohydrates (aNDFom) and non-ammonia crude protein (CP).

Dietary component	Chemical fraction	*W* (fraction of DM)	*D* (fraction of DM)	kd (per d)
Grass herbage	CP	0.150	0.775	2.16
	aNDFom	0.000	0.875	1.44
Grass silage	CP	0.350	0.550	1.20
	aNDFom	0.000	0.825	0.96
Maize silage	CP	0.575	0.225	0.48
	aNDFom	0.000	0.600	0.48
	ST	0.300	0.700	2.40
Concentrates standard	CP	0.325	0.625	1.56
	aNDFom	0.000	0.850	1.80
	ST	0.575	0.425	2.40
Concentrates protein rich	CP	0.225	0.750	1.44
	aNDFom	0.000	0.800	1.44
	ST	0.300	0.700	1.92

[a] The model requires input data for rumen *in situ* degradation characteristics of crude protein (CP), starch (ST) and cell wall carbohydrates (aNDFom). A distinction is made between the rumen washable fraction (W), the non-washable, but potentially degradable fraction (*D*) and the non-washable, undegradable fraction (*U*) of CP, ST or aNDFom, with $U = 1 - W - D$. For the *D* fraction, an additional estimate of the fractional degradation rate (kd; per d) of *D* is required.

Table 6
Feed intake (DM[a] intake and GE[a] intake), and milk production for the average dairy cow in The Netherlands (CBS, 2009; www.cbs.nl), with estimated enteric methane emissions.

	DM intake (kg /cow/yr)	GE intake (MJ/cow/d)	FPCM[a] yield (kg/cow/d)	MEF[a] (kg/cow/yr)	MCF[a] (fraction of GE intake)	CH_4/FPCM (g/kg FPCM)
1990	5532	280	17.17	110.5	0.0603	17.6
1991	5570	281	17.36	111.2	0.0604	17.6
1992	5574	281	17.59	111.9	0.0607	17.4
1993	5702	288	18.02	113.9	0.0603	17.3
1994	5823	295	18.51	115.6	0.0598	17.1
1995	5779	293	18.91	115.8	0.0602	16.8
1996	5765	292	19.17	113.5	0.0592	16.2
1997	5875	297	19.47	117.0	0.0600	16.5
1998	5953	302	19.68	116.9	0.0590	16.3
1999	5976	303	19.91	119.1	0.0600	16.4
2000	6069	307	20.76	120.0	0.0597	15.8
2001	6141	311	21.05	122.1	0.0599	15.9
2002	6084	308	20.77	120.2	0.0595	15.9
2003	6310	319	21.26	123.3	0.0590	15.9
2004	6356	321	21.50	124.8	0.0593	15.9
2005	6354	320	21.89	126.3	0.0602	15.8
2006	6474	327	22.38	127.8	0.0596	15.6
2007	6591	333	22.82	129.4	0.0592	15.5
2008	6571	332	22.89	128.3	0.0588	15.4

[a] DM, dry matter; GE, gross energy; FPCM, fat and crude protein corrected milk; MCF, methane energy conversion factor; MEF, methane emission factor.

The fractional rumen degradation rate of the potentially rumen degradable fraction of dietary DM was calculated from intrinsic rumen degradation characteristics of individual diet components by weighting their fractional degradation rates against their contribution to the potentially degradable fraction in dietary DM.

3. Results and discussion

3.1. National Enteric Methane Inventory

3.1.1. Enteric methane from 1990 till 2008

Model simulations were completed to predict the rate of CH_4 production in the rumen and large intestine. Predicted MEF ranged from 110.5 to 129.4 kg CH_4/cow/yr and predicted MCF from a fraction of 0.0588 to 0.0607 of GE intake (Table 6). Average MCF from 1990 to 2008 (*i.e.*, 0.0597) is very close to the IPCC (1997) Tier 2 default value of 0.060. However, the predicted value for the average dairy cow in The Netherlands is 9% lower than the MCF value of 0.065 suggested by IPCC in 2006. Compared to the IPCC (1997) Tier 1 default values of a MEF of 118 kg CH_4/cow/yr at a milk yield of 6700 kg of milk/cow/yr (*i.e.*, 17.6 g CH_4/kg milk), comparable values were predicted in 1997 (*i.e.*, 117 kg CH_4/cow/yr or 6803 kg of milk/cow/yr; 17.2 g CH_4/kg milk which is 2% less than the IPCC default). From 1990 onwards, a small decline in MCF was predicted (MCF = 0.0603–0.000065 (year – 1990); $R^2 = 0.45$), likely related to the slightly increased proportion of maize silage, and decreased proportion of grass herbage, in the diet and increased feed intake levels per cow from 1990. This corresponds to the general view on DM intake affecting MCF (Ellis et al., 2008), that when CH_4 is expressed per unit milk produced, its emission declined from 17.6 g CH_4/kg FPCM in 1990 to 15.4 g CH_4/kg FPCM in 2008.

Results indicate that MEF increased with 1.05 kg CH_4/cow/yr or a total increase from 1990 until 2008 of 17% (MEF = 109.86 + 1.05 (year – 1990); $R^2 = 0.96$). Thus, compared to the increase in DM intake (20%) and milk production (34%), CH_4 emission increased at a lower rate (Fig. 4B), leading to a reduction in CH_4 emission of 0.124 g CH_4/kg FPCM/yr ($R^2 = 0.91$) with a total reduction of 12.7% from 1990 until 2008.

3.1.2. Accuracy of methane estimates

The 1997 IPCC guidelines require estimates on uncertainty of MEF and MCF with use of the Tier 3 approach. Therefore, model calculations were completed to investigate effects of error in model inputs and error in model representation on predicted MEF and MCF. An indication might be obtained from published sensitivity analysis of the rumen fermentation model (excluding CH_4) by Dijkstra et al. (1992), Neal et al. (1992), Bannink and De Visser (1997a) and Bannink et al. (1997b,c,d). Mills et al. (2001) analysed simulated effects on enteric CH_4 emission of an increase of DM intake, and of an exchange of grass silage with concentrates or maize silage. Model evaluations against independent data have shown that CH_4 is predicted with higher accuracy than various empirical models (Benchaar et al., 1998; Kebreab et al., 2008) using a predecessor of and the same model as described by Mills et al. (2001), respectively. However, none of the previously mentioned studies evaluated a realistic type and size of error associated with use of diet and cow national statistics as model inputs, as needed by IPCC (1997) guidelines. Thus, our study included an error analysis by evaluating effects of error in model inputs and in

some key model parameters and internal model calculation rules on predicted MEF and MCF. This provided an indication of uncertainty in MEF and MCF to be considered in the National Inventory of GHG emission for the Tier 3 approach.

3.1.2.1. Uncertainty related to model inputs. Data used for the National Inventory Report of 2008, for 2006, were used as a starting point and will be indicated hereon by the term 'reference'. The diet for this reference was composed (DM basis) of 10% grass herbage, 39% grass silage, 26% maize silage, 22% concentrates and 3% wet by-product feeds on a DM basis. For this reference diet, MEF and MCF were 129.4 kg CH_4/cow/yr 0.0591 of GE intake, respectively.

An error analysis was first conducted for feed intake as a model input. The estimated NE_l value of a ration may be too high or too low and directly affect the estimated DM intake of an average dairy cow needed to meet her NE_l requirements. Furthermore, partitioning of energy intake over individual dietary components may be inaccurate, and various scenarios were studied of exchange between some important dietary components. Finally, the chemical composition of the dietary components may be inaccurate, and scenarios were tested with an exchange of some important chemical fractions.

Table 7 indicates effects of these potential inaccuracies on predicted MEF and MCF. Inaccuracies tested were: no correction for feed losses (assumed to be 5, 3 and 2% respectively for ensiled forages, wet by-product feeds and concentrates; CBS, 2009), a 2% inaccuracy in total DM intake, an inaccurate DM intake with either grass herbage or grass silage both at 10% of DM of total of grass products in the reference diet, and an inaccurate DM intake of either maize silage or concentrates, both at 5% of DM intake of maize silage or concentrates, respectively, in the reference diet. Because grass herbage intake is calculated by the WUM (CBS, 2009) as the remainder of NE_l requirement that is not covered by the other known dietary components, all errors with the other dietary components accumulate into error of estimated grass herbage intake. For this reason, the error for estimated grass herbage intake was considered to be equal to that of grass silage intake. Results (Table 7) indicate that an error in estimates of feed losses and error in estimated DM intake of total diet or grass products (as a result of error in estimated energy value) most affected predicted MEF and MCF. Predicted MEF was most affected (4.5% change in MEF value) by error in grass herbage intake, whereas MCF was affected most (1.1% change in MCF value) by error in grass silage intake.

Second, the consequences of erroneous allocation of NE_l requirement to individual dietary components were tested by exchanging 10% of dietary DM between grass herbage and the combination of grass silage, maize silage and concentrates, between grass herbage and grass silage only, between maize silage and grass herbage and grass silage, and between concentrates and grass herbage, grass silage and maize silage (exchange of multiple dietary components was performed according to their proportion in dietary DM). Results indicate (Table 7) that inaccurate estimation of grass herbage against the remainder of dietary DM causes the largest error in predicted MEF (0.9%) and MCF (1.0% of MCF value), but the error was relatively small.

Third, the effect of error in chemical composition of the dietary components was tested. Sugars (20 g/kg DM of grass herbage or grass silage) were exchanged with aNDFom. The exchange was on the analysed fractions of SU and aNDFom, followed by allocation of the fraction not accounted for by feed analysis for 50% to SU and 50% to aNDFom. The same exchange was tested in combination with an exchange of 10 g of CP/kg of DM of grass herbage or grass silage. In case of additional exchange with the CP fraction, the NE_l value of grass herbage or grass silage was also corrected based on historical data, and DM intake of grass herbage or grass silage changed accordingly to achieve an identical NE_l intake. The NE_l value of grass herbage was assumed to range from 945 to 1000 with a CP content ranging from 170 to 230 g CP/kg DM. The NE_l value of grass silage was assumed to range from 860 to 895 g/kg DM, with CP ranging from 150 to 180 g CP/kg DM. For both grass products, data were derived from historical data in Tables 2–4, which indicates the variation in the national average for grass products and subsequent error that can occur. The estimated size of the error was thus based on variation in these historical data. For maize silage, an error in ST content was considered by an exchange of 25 g ST/kg DM against aNDFom. Results indicate (Table 7) that exchange of SU with aNDFom in grass silage in combination with a reduced CP content has the largest effect on predicted MEF (1.0%) and MCF (0.7% of MCF value).

3.1.2.2. Uncertainty related to model representation. Besides model inputs derived from diet and cow national statistics, uncertainty in MEF and MCF is also associated with some key internal model parameters and calculations. Based on previous sensitivity analyses (Neal et al., 1992; Bannink and De Visser, 1997a), key parameters include the rumen fractional passage rates of particles and of fluid, rumen fluid volume and rumen pH. Effects of inaccuracy in these parameter values was evaluated by changing their values respectively by 0.1/d, 0.2/d, 10 L and 0.1 pH unit. Internal calculations considered most subject to error are those related to stoichiometric coefficients of VFA production from fermented substrates (Bannink et al., 1997b). Consequences of erroneous VFA stoichiometry was studied by exchanging the VFA stoichiometry of Bannink et al. (2008, 2010) with that of Bannink et al. (2006) and by that of Murphy et al. (1982). The effects of ignoring the contribution of fermentation in the large intestine was investigated by excluding its contribution to predicted MEF and MCF. Finally, some alterations of the representation of rumen fat metabolism were studied and a mechanism was introduced into the model (not being an element in the Tier 3 model; 2.1.1.1.), according to the approach of Dijkstra et al. (2000) describing effects of fat addition and of degree of saturation of fatty acids on rumen fermentation.

Results in Table 7 indicate that of all model parameters evaluated, error in rumen acidity had the largest effect on estimated MEF and MCF. A reduction of rumen pH by 0.1 units deceased both MEF and MCF by more than 3%. A reduction of pH resulted in a reduction of aNDFom degradation in the rumen and a shift towards higher production of propionic acid from ST and SU leading to a decrease in CH_4 production. Alternative model representations of VFA stoichiometry had the

Table 7
Summary of uncertainty in predicted MEF (kg CH_4/cow/yr) and MCF (CH_4 energy as fraction of gross energy intake) as a result of uncertainty in model inputs and internal model parameters and calculation rules. Averages are given of absolute change in MEF and MCF obtained with uncertainty in positive and negative direction. Signs indicate direction of change with the indicated direction of uncertainty tested.

Prediction for reference year 2006[a]		MEF (kg CH_4/cow/yr) 129.4[a]	MCF (fraction of GE intake) 0.0591[a]
Aspect changed with respect to reference	Size of uncertainty or choice of alternative representation	% change of predicted	
Feed intake (*DM and VEM[b] intake altered compared to reference*)			
Feed losses (no correction for feed losses)	+3.8% DM intake according to WUM	+2.6%	−0.3%
DM intake ration (equal dietary composition)	+2% DM intake	+1.6%	−0.4%
DM intake grass herbage (unequal dietary composition)	+10% of DM intake of all grass products (5.2% total DM intake)	+4.5%	−0.6%
DM intake grass silage (unequal dietary composition)	+10% of DM intake of all grass products (5.2% total DM intake)	+4.0%	−1.1%
DM intake maize silage (unequal dietary composition)	+5% of DM intake maize silage (1.3% total DM intake)	+0.9%	−0.5%
DM intake concentrates	+5% of DM intake concentrates (1.0% total DM intake)	+0.8%	−0.1%
Estimate of total contribution to uncertainty[c]		*5%*	*1.5%*
Partition dietary components (*after DM replacement, DM intake altered to maintain equal VEM[b] intake compared to reference*)			
VEM intake grass herbage (grass and maize silage and conc replaced)	+10% total DM intake	+0.9%	+1.0%
VEM intake grass herbage (grass silage replaced)	+10% total DM intake	+0.4%	+0.9%
VEM intake maize silage (grass herbage and silage replaced)	+10% total DM intake	−0.7%	−0.7%
VEM intake conc. (grass herbage and grass and maize silage replaced)	+10% total DM intake	−0.4%	+0.2%
Estimate of total contribution to uncertainty[c]		*1%*	*1%*
Composition dietary components			
Sugar grass herbage (exchange cell walls)	+20 g sugar/kg DM grass herbage	+0.1%	+0.2%
Sugar&CP grass herbage (exchange cell walls)	*In addition* +10 g CP/kg DM grass herbage	+0.2%	+0.2%
Sugar grass silage (exchange cell walls)	+20 g sugar/kg DM grass silage	+0.5%	+0.5%
Sugar&CP grass silage (exchange cell walls)	*In addition*, +10 g CP/kg DM grass silage	+1.0%	+0.7%

Table 7 (*Continued*)

Prediction for reference year 2006[a]		MEF (kg CH_4/cow/yr) 129.4[a]	MCF (fraction of GE intake) 0.0591[a]
Starch maize silage (exchange cell walls)	+25 g starch/kg DM maize silage	+0.0%	+0.4%
Starch concentrates (exchange sugars)	+50 g starch/kg DM concentrates	−0.5%	−0.5%
Starch&CP concentrates (exchange sugar and CP)	*In addition*, + 10 g CP/kg DM concentrates	−0.1%	−0.2%
Starch concentrates (exchange cell walls)	+50 g starch/kg DM concentrates	−0.1%	−0.0%
Estimate of total contribution to uncertainty[c]		*2%*	*2%*
Model parameters			
Particle passage	+0.1/d	−1.1%	−1.1%
Fluid passage	+0.2/d	−0.6%	−0.6%
Rumen volume	+10 L	+1.0%	+1.0%
Acidity	−0.1 pH unit	−3.1%	−3.1%
Estimate of total contribution to uncertainty[c]		*5%*	*5%*
Internal calculation rules			
VFA-stoichiometry Bannink 2006	Alternative stoichiometry forage rich diets	+10%	+10%
VFA-stoichiometry Murphy 1982	Alternative stoichiometry forage rich diets	+18%	+18%
Estimate of total contribution to uncertainty[c]		*5%*	*5%*
No large intestine	Neglecting large intestine contribution	−8%	−8%
Fat content	Fat content concentrates + 10 g/kg DM	−1.2%	−0.8
Fat saturation	Degree of saturation fatty acids −5%	−0.1%	−0.1%
Acidity and fat	−0.1 pH unit combined with fat content	−3.1%	−3.1%
Estimate of total contribution to uncertainty[c]		*1%*	*1%*
Summation of all total uncertainties		19%	16%
If normal distributed and totally independent		9%	8%
Intermediate uncertainty (best estimate)		15%	13%

[a] The reference diet for prediction of basal values of MEF and MCF differs from Van der Maas et al. (2010) in Table 6. In 2009 MEF and MCF were recalculated for the period of 1990–2008 due to an erroneous correction in the calculation method used for feed intake (CBS, 2009). Correction of this error led to slightly different values for MEF and MCF for the whole time series and MEF for the year 2006 changed from 129.4 to 127.8 kg CH_4/cow/yr, and MCF changed from 0.0591 to 0.0596. This slight difference of the reference values for year 2006 compared to values in Table 6 had no effect on estimates of uncertainty.

[b] VEM is the unit of net energy for lactation used in the energy evaluation system for ruminants in The Netherlands (Van Es, 1978). A unit of VEM corresponds to 6.9 kJ of net energy for lactation.

[c] Per category of aspects studied, uncertainty in MEF and MCF calculated for the individual aspects will not occur in combination. Hence the estimate of the total contribution of this category to uncertainty in MEF and MCF was a weighed value considered realistic, avoiding duplication of the same uncertainties tested with more than one individual aspect. No sign is indicated for the eventual error estimate of uncertainty because this applies to a positive and a negative direction.

highest effect on MEF and MCF. However, as the VFA stoichiometry in Tier 3 was derived from lactating cow data only, and includes a pH effect (Bannink et al., 2010), the error is likely smaller than the simulated difference when exchanging the VFA stoichiometry of Murphy et al. (1982), based on sheep and cattle, for that of Bannink et al. (2006) which neglected pH. For this reason, a 5% error due to incorrect representation of VFA stoichiometry was considered realistic. Thereby, MEF and MCF are most sensitive to error in this representation. The large intestine contributed 8% of total CH_4 emissions, and its omission from calculations would lead to underestimation of MEF and MCF, but its contribution to error is minor. Also, error involved with rumen fat metabolism appeared to have a minor effect (Table 7).

3.1.2.3. Uncertainty of MEF and MCF. Summarizing results on uncertainty with respect to model inputs discussed in Section 3.1.2.1 (*i.e.*, uncertainty of feed intake, ration composition or chemical composition of ration components) resulted in a change in MEF of 5, 1 and 2%, respectively. The accompanying change in MCF was 1.5, 1 and 2%, respectively. Similarly, an incorrect estimate of model parameters and improper representation of internal model equations resulted in a 5 and 5% change in MEF value, and a 5 and 5% change in MCF value, respectively. Therefore, uncertainty of MEF is determined most by variation in feed intake and in stoichiometry of VFA production, followed by that of acidity of rumen digesta and the chemical composition of the ration. In contrast, uncertainty of MCF is determined most by uncertainty of stoichiometry of VFA production and acidity of rumen digesta, followed by that of chemical composition of the ration and feed intake.

When all uncertainties associated with model inputs, model representation and internal model parameters were considered totally independent and normally distributed, the overall uncertainty of MEF became 9% for MEF and 8% for MCF. The sum of all uncertainties (in Table 7) was 19.0% for MEF and 15.5% for MCF, which was the total uncertainty. The assumption of independence obviously does not hold, but also the total summation would be too high because individual uncertainties only add partially. For this reason, intermediate values of uncertainty were considered more realistic, which resulted in 15% uncertainty for MEF and 13% for MCF. These uncertainties are currently applied in the National Inventory of GHG emissions in The Netherlands (Van der Maas et al., 2010). This uncertainty is 25% less than that of the Tier 2 approach used to estimate enteric CH_4 emission (IPCC, 1997).

3.2. Advantages and limitations of the Tier 3 approach

3.2.1. Advantages

The current Tier 3 model for enteric CH_4 emission in dairy cows has several advantages compared to Tier 2. Because it represents mechanisms of enteric fermentation in more detail, it can be expected to describe more of the variation caused by nutritional and animal factors, resulting in improved prediction accuracy of enteric CH_4 emissions, and provides insight into which factors contribute most to uncertainty of CH_4 estimates. This means it serves as a tool to investigate uncertainties related to prediction of enteric CH_4 emission. Furthermore, the model generated physiological and microbial realism when evaluating various nutritional measures as options to reduce CH_4 emissions. The model predicted rumen degradability and microbial growth, as well as stoichiometry of VFA production in the rumen and large intestine, as a result of fermentation of substrates (*i.e.*, SU, ST, aNDFom, CP). None of these aspects is addressed by a Tier 2 approach. For example, the Tier 3 model was recently used to evaluate effects of grassland management on CH_4 emission in dairy cows fed ryegrass based diets (Bannink et al., 2010). In this respect, the model is much more helpful in formulating and testing hypotheses on effects of diet composition than is a Tier 2 approach, or other approaches not building upon representation of underlying mechanisms. The model also considers effects of DM intake and fermentation conditions in the rumen, which has been demonstrated to have a profound effect on enteric methanogenesis (Ellis et al., 2008).

The Tier 3 model is helpful in estimating enteric CH_4 emission and it may aid in evaluation of policy options by quantifying effects of dietary changes on CH_4 and N emissions by dairy cows. The expected existence of a trade-off between reducing N surplus or N excretion/ha (and related emissions) and enteric CH_4 emissions implies that management options should not be evaluated with generic estimates of CH_4 and N_2O emissions, which are treated as independent, and which are not aimed at addressing underlying causal factors. A reduction of the N surplus/ha is often associated with an overall reduction of total GHG emissions (Schils et al., 2006) because of its impact on N_2O emissions. However, this outcome was obtained under assumptions of generic estimates of CH_4 emission, which have been shown to be inadequate (Ellis et al., 2010) and independency of N surplus/ha. The Tier 3 approach may be used to study such issues, to provide a differentiation of generic estimates of GHG emissions, and to evaluate various policy options, for which use of a Tier 2 approach is less useful.

3.2.2. Limitations

Besides advantages, there are some limitations with the use of the Tier 3 approach. First, applicability of the Tier 3 model is limited because it was not developed from measurements of enteric CH_4 emissions, but was developed based on parameters discussed in Section 3.1.2. The aim of our modelling was to represent the most essential aspects of enteric fermentation and methanogenesis. For this reason, the model was not calibrated against experimental data on enteric CH_4 emissions by cows. However, the model predicted a MCF value very similar to the IPCC (1997) Tier 2 default of 0.06, but lower than the 0.065 used by IPCC (2006). Also, the model prediction for g CH_4/kg milk was very close to the Tier 1 IPCC default (IPCC, 1997).

A further limitation is that uncertainty of predicted enteric CH_4 emission remained large (*i.e.*, 15% uncertainty of MEF may be considered of the same order of magnitude as the uncertainty using a Tier 2 approach), despite that Tier 3 is a complex model which considers many of the mechanisms of enteric fermentation. Furthermore, the evolution of predicted CH_4 emission closely follows trends of DM intake and milk production with only moderate variation. One might argue that such a trend could also have been produced with a more empirical approach with comparable uncertainty. The continuous trends are caused by the relatively small and regular changes in diet and cow statistics as illustrated in Fig. 4. More drastic changes in diet and cow characteristics will cause larger deviations between outcomes obtained with both approaches.

Another limitation of the Tier 3 approach is that it requires detailed data on diet composition and rumen degradation characteristics of feed substrates as input because this data are not routinely collected. As well, estimates need to be based on previous *in situ* rumen incubations and national statistics on diet components. Results of *in situ* rumen incubations for dietary components vary as a result of variation in DM intake, the type of diet consumed and rumen conditions (*i.e.*, cow

characteristics), as well as variation in the dietary components. For this reason, a protocol is used with *in situ* incubation to standardize DM intake, type of diet and type of cow, in an attempt to obtain estimates of rumen degradability of dietary components as a truly intrinsic characteristic, and less affected by *in vivo* variation in DM intake, diet and cow performance. The model used in our study requires such intrinsic characteristics as input, and the ability of *in situ* values to predict *in vivo* values is unknown. In The Netherlands, there is abundant research to allow a sound estimate to be made of national averages of rumen *in situ* degradation characteristics of ST, aNDFom and CP in individual dietary components used in the national cow diet. In cases where such data are not readily available, advantages of the Tier 3 may not outweigh the limitations discussed, and the effort needed to collect the data.

A final, more practical, disadvantage of the Tier 3 approach is its dynamic nature and its far higher complexity than a Tier 2 approach. These features require specialized simulation software to run the model, and they hamper straightforward implementation of the model in tools developed for farm surveys and the National Inventory on GHG emissions. Although use of a less complex Tier 2 is appealing for practical reasons, it is a less feasible approach to explain variation in observed enteric CH_4 emissions and to capture this variation in monitoring methodologies.

4. Conclusions

In contrast to a Tier 2 approach, the Tier 3 approach evaluates specific details of nutritional management because the underlying mechanisms of enteric fermentation are represented dynamically. A wide applicability of this Tier 3 approach is expected because it addresses effects of nutritional details on enteric CH_4 emission. The model provides insight into which factors contribute most to uncertainty and, therefore, it is a quantifying tool that complements experimental data for farm management, cow nutrition and enteric CH_4 emission. This model can be used to evaluate consequences of policies aimed at reducing enteric CH_4 production that affect cow nutrition and production, and is the basis of a Tier 3 approach for the National Inventory of GHG emission in The Netherlands.

Conflict of interest statement

None.

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References

Argyle, J.L., Baldwin, R.L., 1988. Modelling of rumen water kinetics and effects of rumen pH changes. J. Dairy Sci. 71, 1178–1188.
Baldwin, R.L., Thornley, J.H.M., Beever, D.E., 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. J. Dairy Res. 54, 107–131.
Baldwin, R.L., Lucas, H.L., Carbrera, R., 1970. Energetic relationships in the formation and utilization of fermentation end-products. In: Phillipson, A.T., Annison, E.F., Armstrong, D.G., Balch, C.C., Comline, R.S., Hardy, R.S., Hobson, P.N., Keynes, R.D. (Eds.), Physiology of Digestion and Metabolism in the Ruminant. Oriel Press, Newcastle-upon-Tyne, UK, pp. 319–334.
Bannink, A., Smits, M.C.J., Kebreab, E., Mills, J.A.N., Ellis, J.L., Klop, A., France, J., Dijkstra, J., 2010. Simulating the effects of grassland management and grass ensiling on methane emission from lactating cows. J. Agric. Sci. (Camb.) 148, 55–72.
Bannink, A., France, J., López, S., Gerrits, W.J.J., Kebreab, E., Tamminga, S., Dijkstra, J., 2008. Modelling the implication of feeding strategy on rumen fermentation and functioning of the rumen wall. Anim. Feed Sci. Technol. 143, 3–26.
Bannink, A., Kogut, J., Dijkstra, J., France, J., Tamminga, S., Van Vuuren, A.M., 2000. Modelling production and portal appearance of volatile fatty acids in cows. In: McNamara, J.P., France, J., Beever, D.E. (Eds.), Modelling Nutrient Utilisation in Farm Animals. CAB International, Wallingford, UK, pp. 87–102.
Bannink, A., Kogut, J., Dijkstra, J., France, J., Kebreab, E., Van Vuuren, A.M., Tamminga, S., 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating dairy cows. J. Theor. Biol. 238, 36–51.
Bannink, A., De Visser, H., 1997a. Comparison of mechanistic rumen models on mathematical formulation of extramicrobial and microbial processes. J. Dairy Sci. 80, 1296–1314.
Bannink, A., De Visser, H., Klop, A., Dijkstra, J., France, J., 1997b. Causes of inaccurate prediction of volatile fatty acids by simulation models of rumen function in lactating cows. J. Theor. Biol. 189, 353–366.
Bannink, A., De Visser, H., Van Vuuren, A.M., 1997c. Comparison and evaluation of mechanistic rumen models. Br. J. Nutr. 78, 563–581.
Bannink, A., De Visser, H., Dijkstra, J., France, J., 1997d. Impact of diet-specific input parameters on the simulated rumen function. J. Theor. Biol. 184, 371–384.
Benchaar, C., Rivest, J., Pomar, C., Chiquette, J., 1998. Prediction of methane production from dairy cows using existing mechanistic models and regression equations. J. Anim. Sci. 76, 617–627.
CBS (Statistics Netherlands), 2009. Livestock Manure and Nutrients 1990–2008. The Hague, The Netherlands (www.cbs.nl).
CVB, 2007. Tabellen Veevoeding 2007. CVB-reeks nr. 33. Centraal Veevoederbureau, Productschap Diervoeder (Product Board Animal Feed). The Hague, The Netherlands (www.pdv.nl).
Dijkstra, J., Gerrits, W.J.J., Bannink, A., France, J., 2000. Modelling lipid metabolism in the rumen. In: McNamara, J.P., France, J., Beever, D.E. (Eds.), Modelling Nutrient Utilization in Farm Animals. CAB International, Wallingford, UK, pp. 25–36.
Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M., Tamminga, S., 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. Br. J. Nutr. 69, 385–396.
Dijkstra, J., Neal, H.D., St., C., Beever, D.E., France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. J. Nutr. 122, 2239–2256.
Ellis, J.L., Dijkstra, J., Kebreab, E., Bannink, A., Odongo, N.E., McBride, B.W., France, J., 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agric. Sci. (Camb.) 146, 213–233.

Ellis, J.L., Bannink, A., France, J., Kebreab, E., Dijkstra, J., 2010. Evaluation of enteric methane prediction equations for dairy cows used in whole farm models. Global Change Biol. 16, 3246–3256.
France, J., Thornley, J.H.M., Beever, D.E., 1982. A mathematical model of the rumen. J. Agric. Sci. (Camb.) 99, 343–353.
IPCC, 1997. Revised 1996 IPCC Guidelines for National Greenhouse Gas Inventories. Intergovernmental Panel on Climate Change, Bracknell, UK.
IPCC, 2006. 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Prepared by the National Greenhouse Gas Inventories Programme. Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (Eds.). IGES, Hayama, Japan.
Kebreab, E., France, J., Agnew, R.E., Yan, T., Dhanoa, M.S., Dijkstra, J., Beever, D.E., Reynolds, C.K., 2003. Alternatives to linear analysis of energy balance data from lactating dairy cows. J. Dairy Sci. 86, 2904–2913.
Kebreab, E., Johnson, K.A., Archibeque, S.L., Pape, D., Wirth, T., 2008. Model for estimating enteric methane emissions from United States dairy and feedlot cattle. J. Anim. Sci. 86, 2738–2748.
Mills, J.A.N., Dijkstra, J., Bannink, A., Cammell, S.B., Kebreab, E., France, J., 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: model development, evaluation, and application. J. Anim. Sci. 79, 1584–1597.
Murphy, M.R., Baldwin, R.L., Koong, L.J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. J. Anim. Sci. 55, 411–421.
Neal, H.D., Dijkstra St., C., Gill, J.M., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model evaluation. J. Nutr. 122, 2257–2272.
Pitt, R.E., Van Kessel, J.S., Fox, D.G., Pell, A.N., Barry, M.C., Van Soest, P.J., 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. J. Anim. Sci. 74, 226–244.
Schils, R.L.M., Verhagen, A., Aarts, H.F.M., Kuikman, P.J., Šebek, L.B.J., 2006. Effect of improved nitrogen management on greenhouse gas emissions from intensive dairy systems in The Netherlands. Global Change Biol. 12, 382–391.
Smink, W., Van der Hoek, K.W., Bannink, A., Dijkstra, J., 2005. Calculation of methane production from enteric fermentation in dairy cows. FIS-report, FIS, Wageningen, The Netherlands.
Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., De Haan, C., 2006. Livestock's Long Shadow: Environmental Issues and Options. FAO, Rome, Italy.
Tamminga, S., Aarts, F., Bannink, A., Oenema, O., Monteny, G.-J., 2004. Actualisation of N and P excretion by cattle (*in Dutch*). Reeks Milieu en Landelijk Gebied 25, Wageningen, pp. 48 (www.wur.nl).
Van der Maas, C.W.M., Coenen, P.W.H.G., Zijlema, P.J., Baas, K., Van den Berghe, G., Van den Born, G.J., Brandt, A.T., Dröge, R., Guis, B., Geilenkirchen, G., Te Molder, R., Nijdam, D.S., Peek, C.J., Van der Sluis, S.M., 2010. Greenhouse Gas Emissions in the Netherlands 1990–2006. National Inventory Report 2010. MNP report 500080017. Netherlands Environmental Assessment Agency, Bilthoven, The Netherlands.
Van Duinkerken, G., Blok, M.C., Bannink, A., Cone, J.W., Dijkstra, J., Van Vuuren, A.M., Tamminga, S., 2011. Update of the Dutch protein evaluation system for ruminants: the DVE/OEB2010 system. J. Agric. Sci. (Camb.). doi:10.1017/S0021859610000912.
Van Es, A.J.H., 1978. Feed evaluation for ruminants. I. The system in use from May 1977 onwards in The Netherlands. Livest. Prod. Sci 5, 331–345.