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Methane emissions from enteric fermentation in dairy cows, 1990-2008

Background document on the calculation method and uncertainty analysis for the Dutch National Inventory Report on Greenhouse Gas Emissions

A. Bannink

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Abstract

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The Dutch protocol for the national inventory estimates the methane emission of the average Dutch dairy cow based on a Tier 3 approach. A dynamic, mechanistic model is used to represent the enteric fermentation processes, using annual national statistics on feed intake and feed composition as model inputs. Dutch dairy rations are based mainly on roughage (3/4 of dry matter ingested) with a high proportion of grass products (2/3 of roughage dry matter). Between 1990 and 2008, there were continuous increases in dry matter intake, milk production and enteric methane emission. Methane emission ranged from 111 to 129 kg/cow/year, and from 17.6 to 15.4 g/kg fat- and protein-corrected milk. The present study indicates that uncertainties in the feed intake level and the proportion and composition of grass products contribute to the variation in predicted methane emission. In addition, internal model equations also greatly contribute to the uncertainty (representation of rumen acidity and yield of volatile fatty acids). The greatest part of the uncertainty in the methane emission factor (kg methane/cow/year) is determined by the uncertainty in the feed intake and stoichiometry of volatile fatty acid production, while the greatest part of the uncertainty in the methane conversion factor (methane energy as % of gross energy intake) is determined by the stoichiometry of volatile fatty acid production in combination with the acidity of rumen digesta. Although the applicability of national statistics as model inputs can be investigated relatively easily in follow-up studies, physiological research with dairy cattle will be required to fully validate the current internal model equations.

Key words: Methane, dairy cattle, modelling, National Inventory Report (NIR)

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Summary

The Dutch protocol for the national inventory of greenhouse gas emissions requires an annual estimate of the methane (CH_4) emission from dairy cows based on a Tier 3 approach. This approach comprises the estimation of CH_4 emission on the basis of national statistics on dairy cattle nutrition as input for a dynamic, mechanistic model of fermentation processes in the rumen and large intestine of dairy cattle (Dijkstra *et al.*, 1992; Mills *et al.*, 2001; Bannink *et al.*, 2005 & 2010). The present report discusses the methodology adopted to derive model inputs, as well as the recent update of this methodology and the recalculated values of CH_4 emissions by dairy cows between 1990 and 2008, and the accuracy of these estimates. So far, the accuracy of these CH_4 estimations had not been systematically examined. Our study aimed to explore the effect of various uncertainties associated with model input values, model parameters and internal model equations on the accuracy of CH_4 predictions.

Estimated CH_4 emission by dairy cows

CH_4 emission by dairy cows was recalculated using an updated methodology that was applied by the Working group on Unifying Manure and excretion data (WUM; www.cbs.nl) to the whole series from 1990 to 2008. The CH_4 emission factor (MEF, kg CH_4/yr) ranged from 110.5 to 129.4 CH_4 kg/yr, and the CH_4 conversion factor (MCF; CH_4 energy as a percentage of gross energy intake with feed) ranged from 5.88 to 6.07%. Since 1990, there have been continuous increases in feed intake (20%), level of milk production (34%) and CH_4 emission (17%), resulting in a continued reduction of CH_4 per kg of fat- and protein-corrected milk (13%).

Accuracy of CH_4 emission estimates

Model inputs or model parameters were varied in three different ways: (1) by varying the feed intake, based on various aspects that determine feed intake, (2) varying the composition of the ration (exchanging ration components), and (3) varying the chemical composition of ration components. Additionally, some model parameters and internal model equations were varied: (4) parameters for fractional passage rate, fluid volume and acidity of rumen contents and (5) equations that represent the stoichiometry of the yield of volatile fatty acids from fermented substrates, the fermentation in the large intestine, and the rumen fat metabolism. All these variations were used to investigate the effects on predicted CH_4 emission in terms of the changes in the CH_4 emission factor (MEF) in kg of CH_4/dairy cow/year and in the CH_4 conversion factor (MCF) in CH_4 as a percentage of gross energy intake with feed.

The simulation results indicated that inaccuracy in feed intake, ration composition and the chemical composition of ration components resulted in changes in the MEF of 5, 1 and 2%, respectively. De corresponding changes in the MCF were 1.5, 1 and 2%, respectively. Incorrect estimates of model parameters and improper representations by internal model equations resulted in 3 and 6% changes in MEF, and 5 and 6% changes in MCF, respectively. This means that the greatest part of the uncertainty in the MEF is determined by the uncertainty in the feed intake and the stoichiometry of volatile fatty acid production, followed by the uncertainties in the acidity of rumen digesta and the chemical composition of the ration. The greatest part of the uncertainty in the MCF is determined by the uncertainty in the stoichiometry of volatile fatty acid production in combination with that in the acidity of rumen digesta, followed by the uncertainty in the chemical composition of the ration and feed intake. Since a number of the inaccuracies studied are partly interdependent, total inaccuracies of 15 and 13% in the predicted MEF and MCF values, respectively, were considered the most realistic values.

The results indicate that the uncertainties in the internal model equations (stoichiometry of volatile fatty acid and methane yield with substrate fermentation, and acidity of rumen contents) are the most important factors determining the accuracy of MEF and MCF estimates. This uncertainty is independent of the uncertainty in the statistical data serving as model input. Higher accuracy for both aspects can only be achieved by more detailed nutritional research, including rumen physiology.

Uncertainty concerning feed intake affected the predicted MEF to a similar extent as uncertainty in internal model equations, but the predicted MCF far less. Uncertainty concerning feed composition (proportion of ingredients and chemical composition of ingredients) had a relatively small impact on the accuracy of MEF and MCF estimates. The proportion of grass herbage and grass silage appeared to contribute most to this uncertainty. This type of uncertainty is closely connected to the method used to gather the statistical data which serve as the model input. Possibly, a comparison can be made with research on farms in practice, but this then needs to be accompanied by careful recording of the nutrition (intake and composition) and performance (milk production) of dairy cows.

1 Introduction

Emission of methane (CH_4) by cattle, as a result of microbial fermentation in the rumen and large intestine, contributes significantly to the greenhouse effect. Cattle produce by far the largest part of CH_4 emitted by agriculture, and dairy cattle contribute the most. Expressed in CO_2 equivalents, the contribution of CH_4 from agriculture in the Netherlands is of the same order of magnitude as the contribution of nitrous oxide. For both gases, with agriculture contributing slightly more than 50% of the total national emissions of both gases (Van der Maas *et al.*, 2010). In view of the large contribution to CH_4 emissions by dairy cows, and since CH_4 emissions vary with feed intake and diet composition, enteric CH_4 in dairy cows is estimated each year using a Tier 3 approach, according to the Dutch protocol for the national Emission Registration (ER; Van der Maas *et al.*, 2010).

In the current IPCC guidelines (1996), a Tier 2 approach uses a default value for the CH_4 conversion factor of 6.0% of gross energy (GE) intake by dairy cows. The type of diet strongly determines, however, whether this 6.0% is accurate. Moreover, in view of the variation in the composition and quality of the diet, and in feed intake and milk production levels, it is not likely that the fraction of GE emitted as CH_4 has been constant between 1990 and 2008. For this reason, a Tier 3 approach was chosen in the Netherlands to estimate enteric CH_4 emission by dairy cows, which enables the characteristics of microbial fermentation processes in the gastrointestinal tract of the cow to be taken into account. A mechanistic, dynamic model is used to represent the microbial fermentation processes in the rumen and large intestine, and model input data are obtained from national statistics on diet composition, diet quality, feed intake and milk production levels achieved.

This report describes the Tier 3 approach to estimate enteric CH_4 emission by dairy cows in the Netherlands, and the calculations that have been performed for the national ER. The Tier 3 approach calculates year-specific values of the CH_4 emission factor (MEF) expressed in kg CH_4/cow/year, and the CH_4 conversion factor (MCF) expressed as a percentage of the GE intake by dairy cows.

The report first describes the results of a recalculation of MEF and MCF which had to be carried out for the period from 1990 to 2008, as a consequence of a revision of historic data on feed intake by dairy cows (Van Bruggen, 2010; www.cbs.nl). This series of estimates was flawed in that the occurrence of feed losses was not taken into account, leading to an exaggerated estimate of feed intake. Simultaneously, the methodology applied to calculate feed intake was found not to have been uniform for the whole 1990 to 2008 period, and the methodology was therefore updated applying a single fully uniform method of calculation to the whole period. Both methodology changes led to an higher estimate of feed intake for the 1990 to 2002 period, and a lower feed intake from 2003 onwards, compared to the feed intake estimates used for previous Emission Registrations for the period from 1990 to 2008. In addition to these recalculations for this period, the more recent estimates of MEF and MCF for 2007 and 2008 have also been included in the report.

The report continues with the results of an analysis of the uncertainties in MEF and MCF estimates. Application of a country-specific Tier 3 approach requires estimation of the uncertainty in the results obtained. The goal of this part of the study was to determine of the order of magnitude of the uncertainty in the estimated CH_4 emissions by dairy cows. We report on the calculations used to identify the uncertainty that needs to be taken into account in the national ER. The results provide an indication of the consequences of the uncertainty in model inputs, some important internal model parameters and some internal calculation rules for the uncertainty in the MEF and MCF estimates used for the ER.

2 The Tier 3 approach in the Dutch protocol

Considering the contribution of dairy cows to the total national CH_4 emission in the Netherlands, an accurate estimation of rumen and large intestinal fermentation in dairy cows is of importance for the ER. Compared to the Tier 2 approach, the Tier 3 approach is thought to deliver a more accurate and a more country-specific indication of the trend of enteric CH_4 emission to be expected as a result of developments in management on Dutch dairy farms. Enteric CH_4 emission is estimated with a mechanistic, dynamic model of enteric fermentation processes in the dairy cow. Estimates are obtained by making use of data on feed intake level and feed composition of dairy cattle gathered in national inventories.

2.1 Inputs from national inventory on nutrition and performance of dairy cows

This section describes of the Tier 3 approach for estimation enteric CH_4 emission in dairy cows, of the data used as an input to this model, and of the assumptions made. A more detailed explanation of the elements represented in the Tier 3 model, and of the features of this model will be presented in Section 2.2 and 2.3. A technical description of the inputs required by the model, a description of required model inputs in Section 2.4, and the recently updated calculations on MEF and MCF values for dairy cows in the period of 1990 till 2008 in Section 2.5.

According to the current protocol for calculation of CH_4 emission in dairy cows in the ER, national statistics nutrition and performance of dairy cows are used. These data are delivered by the Working group Unifying Manure and excretion data (WUM) on yearly basis and the data are gathered by a national inventory performed by the Central Bureau for Statistics (CBS; Van Bruggen, 2010; www.cbs.nl). The following data are gathered in their inventory:

- the number of dairy cows;
- registered national milk production;
- a weighed yearly average of feed intake;
- a weighed yearly average of diet composition;
- data on feed analysis and chemical composition of forages, and roughly the composition of compound feeds (concentrates).

For a description of details on model inputs and data gathered by the CBS and WUM, the reader is referred to Appendix 1 & 2. Recently, there has been a revision of the methodology. Feed losses are taken into account, and the method of calculating feed requirements for the average Dutch dairy cow was revised. This revised methodology was applied to the period of 1990 till 2008 in order to have a uniform method applied for the whole time series, and enteric CH_4 emission was recalculated (described in Section 2.5 and Appendix 1).

2.2 Assumptions

Some assumptions had to be made to transfer the data delivered by the national inventory by WUM and CBS (www.cbs.nl) to inputs for the Tier 3 model.

1. Allocation of dry matter not accounted for

Dietary dry matter (DM) not accounted for by the standard analysis of the fractions of ash, sugars, starch, Neutral Detergent Fibre (NDF), crude protein, crude fat or organic acids (mainly fermentation products in silages) has to be attributed to organic matter. This organic matter unaccounted for was allocated for 50% to sugars and 50% to NDF with products which are relatively rich in sugar and NDF (grass products and some by-products), and for 50% to starch and 50% to NDF with products relatively rich in starch (maize silage, concentrates).

2. Feed losses

The feed intake by dairy cows reported by WUM and CBS (www.cbs.nl) is calculated by taking into account a loss of 0, 5, 3 and 2% of the amount of resp. grass herbage, silage (grass silage and maize silage), byproducts and concentrates (standard and protein-rich concentrates) offered to cows.

3. Chemical composition dietary ingredients

The data on chemical composition of roughages (grass herbage, grass silage, maize silage) are gathered by consultation of the databases of the main commercial laboratory data in the Netherlands performing such analyses (BLGG; www.blgg.nl).

Data on the type and the amount o byproducts and concentrates fed to dairy cattle are collecting by CBS after consulting the feed industry. The chemical composition of byproducts is derived from feeding tables and for concentrates the crude protein content is obtained by consulting feed industry whereas other details are derived from feeding tables. For concentrates a dry matter content of 87% was assumed.

The NDF content in grass herbage in year 2004, 2005 and 2006 was derived from the value applied by Smink *et al.* (2005) for year 2003 taken as a reference value (432 g NDF/kg DM). The fraction of DM unaccounted for the analysed chemical fractions was subsequently allocated to the sugar and NDF for 50% each. However, since the year NDF content is available from the analysis by the commercial laboratory.

4. Correction of crude protein content for ammonia-N

The content of crude protein in dietary DM cannot be used directly as an input to the Tier 3 model because ammonia-N and protein are separate inputs in the model. Therefore, a distinction needs to be made between ammonia-N and the remainder of N in crude protein. This distinction is also applied in the methodology for the national inventory on nutrition and performance of dairy cows by WUM and CBS (www.cbs.nl).

2.3 Mechanistic, dynamic model of methane production

The current model of CH_4 production is derived from the model of rumen fermentation developed by Dijkstra *et al.* (1992). The advantage of the model in comparison to other methods of calculation of rumen fermentation is that it represents the mechanisms underlying the microbial degradation of feed particles. Calculated degradation of feed particles and of the formation of end-products of fermentation (microbial mass, volatile fatty acids (VFA), ammonia, CH_4 and CO_2) are functions of rumen concentration of feed particles, micro-organisms, and of fermentation conditions existing in the rumen. These conditions include the dynamics of acidity of rumen fluid, the dynamics of rumen outflow of fluid and particles, and rumen fluid volume.

The rumen model was extensively evaluated by Dijkstra *et al.* (1992) and Neal *et al.* (1992), and subsequently by Bannink *et al.* (1997a, 1997b). Based on these evaluation results, a new representation was derived for the amount and the type of VFA formed as end-product of rumen fermentation by analysis of a data base of in vivo data from lactating cows (Bannink *et al.*, 2000, 2006). These results were subsequently used by Mills *et al.* (2001) when adapting the rumen model for making it applicable to calculate CH_4 production in the rumen and large intestine of dairy cows. A representation of hydrogen balance was added to the rumen model, and CH_4 production was calculated from the hydrogen surplus ((the required CO_2 by methanogens is always available in excess and not the determinant factor for the quantity of CH_4 produced). Furthermore, a representation of fermentation processes in the large intestine was added to the model. A crucial assumption is that the net production of hydrogen is completely used for CH_4 production. The model also neglects other possible sinks of hydrogen, such as saturation of unsaturated long chain fatty acids or sulphate reduction (see Ellis *et al.*, 2008). The amount and the type of VFA formed is most determinant for hydrogen surplus and the amount of CH_4 generated from this.

Since 2005, the representation of VFA production was improved by making production of VFA from fermented non-structural carbohydrates dependant on the acidity of rumen fluid, en by deriving separate VFA-relationship for roughage-rich and for concentrate-rich diets (Bannink & Dijkstra, 2005; Bannink *et al.*, 2008 & 2010). The VFA-relationships were derived from a database of in vivo observations of the amount of soluble carbohydrates, starch, hemi-cellulose, cellulose and protein digested in the rumen, of rumen fluid acidity, of rumen fluid volume and of the concentration of individual VFA in rumen fluid.

A direct applicability of the VFA-relationships to predict CH_4 production in dairy cows with the current model is plausible. Three important arguments can be given. First, rumen fermentation conditions are now taken into account to estimate VFA production. In a simulation study Bannink *et al.* (2005) demonstrated the effect of fermentation conditions on predicted amounts of CH_4 formed. The results correspond to the effects observed in vivo in dairy cows (Bannink, 2007). Second, the relationships are fully based on in vivo observations in the rumen of lactating dairy cows only. Alternative approaches to represent VFA in literature often made use of in vitro results (Argyle & Baldwin, 1988; Pitt *et al.*, 1996; Van Laar & Van Straalen, 2004). In vitro VFA-profiles are not a good measure of the effects on VFA observed in vivo however (Dijkstra *et al.*, 2005). Third, the relationships are fully derived from observed effects in the rumen in vivo and were derived with a regression model which represented the fermentation of feed substrate, VFA production and VFA-clearance from the rumen. The choice was made to have the regression model approach the representation in the rumen model as close as possible. Apart from the distinction between roughage-rich (more than 50% roughages in dietary DM) and concentrate-rich diets (more than 50% concentrates in dietary DM), the regression model did not contain other factors to explain the relationship between VFA production and ration in an empirical manner. Such an approach was used by Sveinbjörnsson *et al.* (2006). They followed the same approach as that of Bannink *et al.* (2000) but made the representation of VFA production in the regression model dependant as well on some general dietary characteristics. This approach hampers application of these relationships in the rumen model, and makes them less applicable for different production conditions. For a detailed discussion of the different approaches that have been published in literature, the reader is referred to a recent review by Dijkstra *et al.* (2008a).

The model applied in the Tier 3 approach is a mechanistic, dynamic model which represents the underlying fermentation mechanisms in the rumen. Predicted CH_4 production hence is not a result of assumption on rumen CH_4 production (by inserting internal model parameter values or by adding estimates to the model during model development). It is in contrast an outcome of the model as a result of the predicted effect of nutrition on microbial activity, VFA production and the belonging hydrogen surplus and belonging CH_4 production. This is a crucial difference with most other model approaches and calculation methods, and also essentially different from the Tier 2 approach which adopts a constant MCF value (Table 1A versus Table 1B). As part of the Tier 3 approach the model this value in dependency from feed intake, diet characteristics and rumen fermentation conditions (acidity, fluid volume, fractional passage rates of fluid and particulate matter).

Figure 1 gives a schematic representation of the distinction between the Tier 2 and Tier 3 approach. The rumen fermentation conditions can be given as an input to the model based on experimental findings, but can also be predicted by the empirical regression equations described by Mills *et al*. (2001). With formulation of the Dutch Tier 3 approach the choice has been made for the latter option. The model also requires inputs on the degradation characteristics of protein, starch and cell wall material (the washable fraction W, the non-washable but degradable fraction D, the undegradable fraction U and the fractional degradation rate kd that applies to the D fraction). The WUM-data do not give an estimate for these characteristics, however, and the following estimates were made based on previous research by ASG (Table 2). The fraction W, D and U (equal to 100-W-D) indicate resp. the washable, the potentially degradable and the undegradable fraction of protein, starch or cell walls (%), and kd indicates the fractional degradation rate (%/h) of D.

*Table 1A. Overview of the input data required for the **Tier 2** approach.*

Milk production	Net energy (VEM) intake [1]	GE-intake [1]
Growth, gestation, etc.	Net energy value of the diet [1]	MCF [2]

*Table 1B. Overview of the input data required for the **Tier 3** approach (for a more detailed explanation see Dijkstra et al., 1992, and Mills et al., 2001).*

Chemical composition of the diet	**Additional parameter inputs**
Soluble carbohydrates/sugars (g/kg DM)	Feed intake (kg DM/d) [1]
Degradable starch (g/kg DM)	Fractional passage rate particles (/d) [3]
Soluble starch (g/kg DM)	Fractional passage rate fluid (/d) [3]
Cell walls (NDF) (g/kg DM)	Fluid volume (L) [3]
Degradable cell walls (NDF) (g/kg DM)	
Fraction cellulose in cell walls	Average pH [3,4]
Nitrogen / crude protein (g N/kg DM)	Minimum pH [3]
Soluble protein (g/kg DM)	Time period with pH<6.3 (h) [3]
Undegradable protein (g/kg DM)	
Ammonia Nitrogen (g NH_3-N/kg DM)	Fractional degradation rate protein (/d)
Fat (g/kg DM)	Fractional degradation rate NDF (/d)
	Fractional degradation rate starch (/d)

[1] The Tier 2 and Tier 3 approaches both require information on diet composition and feed intake.

[2] The IPCC guidelines (1996) use a default value for MCF of 6.0% of the GE intake, for high-yielding lactating cows, and this value is in fact a fixed assumption in the calculations on CH_4 emissions. This value may need to be differentiated for various production conditions, however. In this respect, it has been suggested within IPCC to change the default MCF value in the Tier 2 approach from 6.0% to 6.5%. In the Tier 3 approach described in the present report, a year-specific value is predicted by the model from input data gathered in national inventory studies on dairy production.

[3] For the rumen as well as the large intestine. In the Tier 3 approach, the value of these parameters is estimated by empirical regression equations and hence not required as an input to the model. For further details see Dijkstra et al. (1992) and Mills et al. (2001).

[4] The average pH value is used in the representation of pH-dependent VFA production rates.

2.4 Inputs required by Tier 3 model

With model prediction of MEF and MCF data on feed and animals are used that have been gathered on a national level. These are the data as being used by the WUM. The data are generated by the Central Bureau for Statistics (CBS) as was described by Smink *et al.* (2005). The data on animal level concern yearly averages of the daily intake of feed (kg DM/cow/day; DM for dry matter) and milk yield (kg fat-corrected milk/cow/day) by the average cow that has been counted in the population of dairy cattle present in the Netherlands. Effects of the dry period and gestation have been taken into account with these data of yearly averages. In establishing the yearly average ration, a distinction is made between grass herbage, grass silage, maize silage, wet by-products, standard concentrates and protein-rich concentrates. The chemical characteristics of grass herbage, grass silage and maize silage are derived from analytical results gathered by the laboratory Bedrijfslaboratorium voor Grond- en Gewasonderzoek (BLGG; www.blgg.nl), those of concentrates and wet by-products from data delivered by feed manufacturers and suppliers of wet by-products. Table 1 gives a comparison of input data required for the Tier 2 approach and for the model in the Tier 3 approach, which have to be generated by inventory studies of nutrition and production of the average Dutch dairy cow.

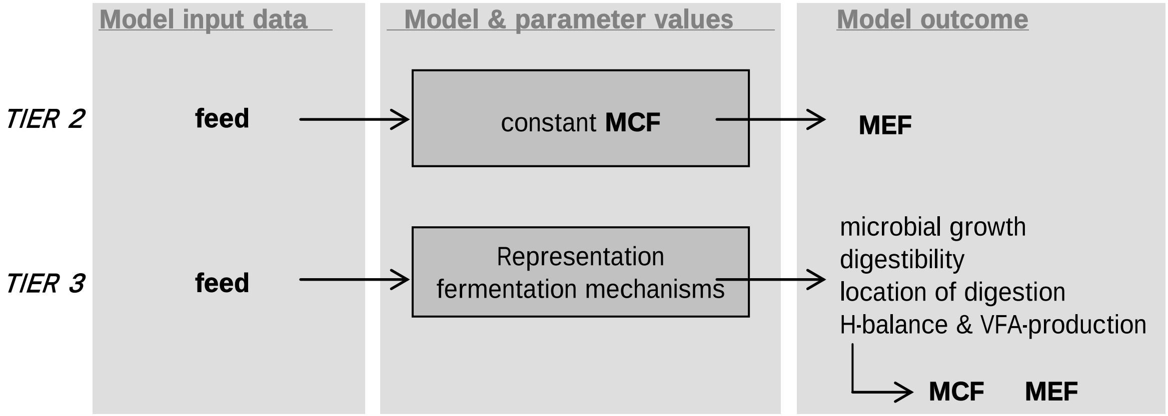

Figure 1. Schematic representation of the difference between the calculation rules (indicated by the grey frames) used in the Tier 2 approach (according to IPCC, 1996) and those used in the Tier 3 approach (according to Mills et al., 2001, and Bannink et al., 2008) to determine the MEF (kg CH$_4$/cow/year) and MCF (CH$_4$ as % of the GE intake).

Table 2. Overview of the assumptions made about in situ rumen degradation characteristics of protein, cell wall material and starch in the various dietary components.

	Protein			**Cell walls**			**Starch**		
	W	**D**	**kd**	**W**	**D**	**kd**	**W**	**D**	**kd**
Grass herbage	15.0	77.5	9	0.0	87.5	6	*NA* [1]		
Grass silage	35.0	55.0	5	0.0	82.5	4	*NA* [1]		
Maize silage	57.5	22.5	2	0.0	60.0	2	30.0	70.0	10
Standard concentrates	32.5	62.5	6.5	0.0	85	7.5	57.5	42.5	10
Protein-rich concentrates	22.5	75.0	6	0.0	80	6	30.0	70.0	8

[1] *NA: Not applicable because starch fraction is absent.*

2.5 Enteric CH_4 emission in dairy cows (1990 till 2008)

According to the revised methodology to derive the input data required by the Tier 3 approach of enteric CH_4 emission in dairy cows, a recalculation was performed of CH_4 emission for 1990 till 2008. The input data derived from national inventory studies by the WUM are listed in Appendix 1. The results are listed in Table 3 and the linear trends indicate that for the average Dutch dairy cow in the period of 1990 till 2008 feed intake increased by 20%, production of fat and protein corrected milk increased by 34% and MEF increased by 17%. Hence, both feed intake and milk production increased at a faster rate than enteric CH_4 emission, leading to a 13% reduction of enteric CH_4 per kg of fat-corrected milk (Figure 2).

Table 3. Feed dry matter (DM) intake (kg DM/cow/yr), ***GE*** *intake (MJ/cow/yr), enteric CH_4 emission (MEF, kg CH_4/cow/yr; MCF, CH_4 energy as % of GE intake) and production of fat- and protein-corrected milk (FPCM) (kg FPCMcow/d)*

Year	Feed intake		Milk production	Methane		
	kg DM/cow/yr	**MJ/cow/d**	**kg FCM/cow/d**	**MEF kg/cow/yr**	**MCF % of GE intake**	**g CH_4/kg FPCM[1]**
1990	5532	280.17	17.17	110.5	6.03	17.62
1991	5570	280.53	17.36	111.2	6.04	17.55
1992	5574	281.07	17.59	111.9	6.07	17.44
1993	5702	288.17	18.02	113.9	6.03	17.32
1994	5823	294.87	18.51	115.6	5.98	17.11
1995	5779	292.92	18.91	115.8	6.02	16.77
1996	5765	292.02	19.17	113.5	5.92	16.21
1997	5875	297.18	19.47	117.0	6.00	16.46
1998	5953	301.94	19.68	116.9	5.90	16.28
1999	5976	302.62	19.91	119.1	6.00	16.39
2000	6069	306.52	20.76	120.0	5.97	15.84
2001	6141	310.82	21.05	122.1	5.99	15.89
2002	6084	308.25	20.77	120.2	5.95	15.86
2003	6310	318.68	21.26	123.3	5.90	15.90
2004	6356	320.94	21.50	124.8	5.93	15.90
2005	6354	319.87	21.89	126.3	6.02	15.81
2006	6474	326.79	22.38	127.8	5.96	15.64
2007	6591	332.99	22.82	129.4	5.92	15.53
2008	6571	332.44	22.89	128.3	5.88	15.36

[1] In contrast to the results for the production of fat-corrected milk (FCM), and for g CH4/kg fat-corrected milk presented in Appendix 1, results in this table are expressed as fat- and protein-corrected milk (FPCM). This is the more familiar unit used in the literature on dairy nutrition. Use of FPCM (this table) compared to use of FCM (Appendix 1) results in only minor changes in results.

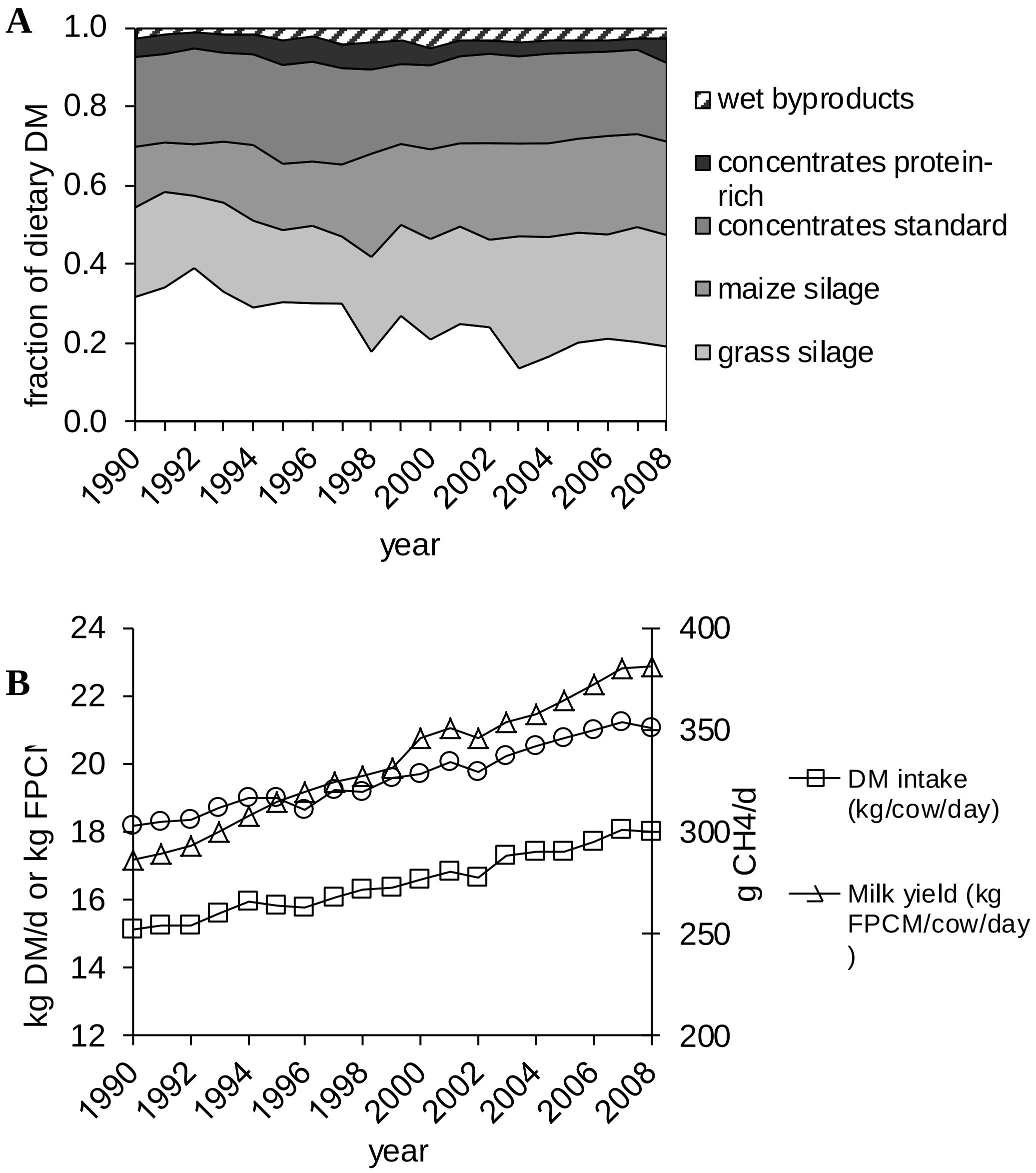

Figure 2. Development of the diet of the average dairy cow in the Netherlands from 1990 to 2008 (www.cbs.nl). (A) Development of dry matter (DM) intake (kg DM/cow/year) and dietary composition, (B) development of DM intake (kg DM/cow/day), Gross Energy (GE) intake (MJ/cow/day) and production of fat- and protein-corrected milk (FPCM; kg/cow/day). The DM in wet by-products was assumed to be composed of 25% brewer's grains, 25% by-products from the potato processing industry, and 50% pressed beet pulp between 1990 and 2003, compared to 25%, 40% and 35%, respectively, between 2004 and 2006; 31%, 42% and 27%, respectively, in 2007; and 32%, 43% and 25%, respectively, in 2008.

3 Uncertainty analysis

This second part of the study was aimed to calculate the effect of the uncertainty around the average ration of the Dutch dairy cow, or of the level of feed intake or milk production, on the uncertainty of predicted MCF and MEF. This uncertainty was established by testing the effects caused by changes in the model inputs, in some model parameters and in some internal calculation rules. The goal of this part was not to explore the potential of nutritional measured to mitigate CH_4 emissions in dairy cows. Another approach would be needed for this, and the independent alteration of the various model inputs could not be performed then. Moreover, the changes in the model input data would of another order of magnitude than is realistic as an estimate of their uncertainty with the national inventory. In the present study the change sin model inputs tested reflect the size of error that might be made in estimating the various model inputs for the ER, and it is by no means a reflection of variation between farms or changes that come along with nutritional measures taken in practice. Analysis of the effect of nutritional measures, or of changed production conditions, or of the effects of regional differences on predicted CH_4 emission in dairy cows were not part of this study.

The uncertainty analysis was carried out before the revision of the methodology for the national inventory of diets and performance of dairy cows (see Chapter 2). Therefore, the calculations of uncertainty were made with the conditions reported for the year 2006 as a reference situation (results for these conditions are described in Appendix 2).

3.1 Approach

The model input data for the ER of the year 2006 (see Appendix 2) have been used as a starting point and will be indicated hereafter by the term ‘reference’. The ration for the reference situation in 2006 was composed of 10% grass herbage, 39% grass silage, 26% maize silage, 22% concentrates and 3% wet by-products on a DM basis (Appendix 2). For this particular ration a MEF of 129.4 kg CH_4/cow/year was calculated and a MCF of 5.91 % accounting for GE-intake. An uncertainty is involved with every input. Uncertainties of model input render uncertainty of predicted MEF and MCF. For various model inputs the size of this uncertainty was investigated:

1. An error analysis was conducted for feed intake as a model input. Estimated value for the net energy of lactation (VEM-value; Van Es, 1978; Tamminga *et al.*, 2004) of a ration may be too high or too low, which means that the average Dutch dairy cow must have achieved a different feed intake (in kg DM/cow/day) to cover the VEM-requirements according to the reference situation (a higher and lower feed intake, respectively).
2. The partitioning of the VEM intake of an average Dutch dairy cow into individual dietary components (grass herbage, grass silage, maize silage, concentrates, wet by-products) may be inaccurate. Various scenarios have been studied with an exchange of some important components against each other.
3. The chemical characterisation of the dietary components grass herbage, grass silage, maize silage and standard concentrates may be inaccurate. Scenarios were studied with exchange of the some important dietary components.

In addition, it was investigated to what extent uncertainty in the model representation contributes to uncertainty of predicted MEF and MCF:

1. Estimates of some strongly determinant internal model parameters may be inaccurate or less applicable for the average Dutch dairy cow. The effects were tested of different assumptions on acidity of the rumen environment, of the fractional passage rate of fluid and of particulate matter, and of rumen fluid volume on predicted CH_4 emissions.
2. The model contains some internal calculation rules that strongly dictate the predicted quantity of CH_4. It concerns, first, the calculation rules for the production of VFA and associated hydrogen balance, and the hydrogen surplus that is converted into CH_4 which emits from rumen and large intestine. Secondly, there is uncertainty with respect to the contribution of the large intestine to total CH_4 emission in dairy cows.
3. Finally, CH_4 production is sensitive for rumen fat metabolism. Altering rumen fat metabolism by dietary measures may affect CH_4 emission in the dairy cow. Model simulations were performed to study to what extent taking into account these dietary effects may contribute to inaccuracy of predicted CH_4 production, or to study the sensitivity for fats that may be fed in the future with the aim to mitigate CH_4. Only for fat such a future perspective was evaluated as a nutritional measure.

3.2 Uncertainty of model input data

Uncertainty of predicted MCF and MEF was determined according to presumed uncertainties of reported values of feed intake, milk production level, and dietary composition and dietary characteristics for the average Dutch dairy cow in the year 2006. The following describes how these uncertainties were investigated.

3.2.1 Feed intake

Feed losses

Feed losses occur in practice. This means that part of the feed offered to dairy cows will actually not have been consumed and hence also cannot have contributed to rumen microbial fermentation and CH_4 production in rumen and large intestine. The WUM (www.cbs.nl) adopts feeding losses of 5% for conserved roughages (grass and maize silages), 3% for wet by-products and 2% for concentrates. The extent at which these losses occur in practice is uncertain (dependant on farm management and farmer). To get an impression of the impact of the presumed feed losses on predicted MEF and MCF, the effect was tested of the reduction of intake of all above mentioned diet components by thee presumed feed losses associated with them (indicated by the term *feed losses*).

Feed intake

Experimental research (under controlled experimental conditions as well as with monitoring of dairy farms in practice) indicates that an error of 5% with estimation of DM-intake and of the VEM-requirement (given milk production) is realistic. For the average Dutch dairy herd it is plausible however that the error is much smaller than in incidental cases. For this reason, an error of 2% was presumed for estimated DM-intake by the average Dutch dairy cow, with identical dietary composition (indicated by the term *DM-intake ration*).

This uncertainty in DM-intake of the whole ration can also be attributed to an individual dietary component, or to a group of dietary components. This was done subsequently with a decrease or increase of DM-intake of grass herbage or grass silage equal to 10% of total DM-intake of all grass products in the reference situation (indicated by the term *DM-intake grass herbage* and *DM-intake grass silage,* respectively; absolute amounts of intake of non-grass components remained unchanged). Uncertainty with respect to the distribution of DM-intake over grass herbage and grass silage is relatively large because intake of grass herbage is calculated as the remainder of total DM-intake after estimation of intake of all other diet components. Moreover, it has been established for grass rations that milk yield is lower than predicted from VEM-intake. The dairy cow hence seems to require more VEM (over 6% more) than according to the VEM-system on diets composed of more than 70% of grass (Dijkstra *et al.*, 2008b). The VEM system also underestimates the VEM-requirement for maintenance of the modern dairy cow, as established by Kebreab *et al* (2003) who compared data from the sixties and seventies (that served as basis for the development of the VEM-system) to more recent data from the nineties. For a large part such effects already have been taken into account by the revision of the methodology used with the national inventory on diet and performance of the average dairy cows reported by WUM (already discussed in Chapter 1 and Chapter 2; www.cbs.nl). All these possible errors with the assumptions on milk yield and VEM-requirement, and with the estimated intake of concentrates, wet by-products, maize silage and grass silage, end up in the estimate of grass herbage intake.

Further, an increase or decrease of DM-intake of maize silage or standard concentrates was tested equal to 5% of DM-intake of maize silage or standard concentrates in the reference situation (indicated by the term *DM-intake maize silage* and *DM-intake concentrates,* respectively; intake of non-maize and other components equal). Uncertainty around availability of maize silage and concentrates is of a smaller size than that of partition of grass intake into that of grass herbage and grass silage. For this reason an uncertainty was assumed of 5% instead of the 10% of DM-intake with DM-intake of grass products.

3.2.2 Distribution over dietary components

Next to uncertainty on the level of DM-intake (and VEM-requirement) also the distribution of feed intake over the various dietary components at equal DM-intake is uncertain. This uncertainty was investigated by the changing the distribution of DM-intake over the individual dietary components (in contrary to the calculation described in Section 3.2.1), while adopting an identical VEM-intake. Because the VEM-value of dietary components differed, after exchange on a DM basis, the DM-intake of the whole diet was corrected to maintain an identical VEM-intake.

The effect was tested of substitution of grass herbage for grass silage, maize silage and concentrates (the latter three in proportion to their contribution to DM in the reference diet) by 10% of Total DM-intake (indicated by the term *VEM-intake grass herbage*). The same type of calculation was performed for substitution of grass herbage for grass silage only (indicated by the term *VEM-intake grass herbage/grass silage*). Maize silage and standard concentrates were exchanged by 5% of total DM-intake. Maize silage was substituted for grass herbage and grass silage (the latter two in proportion to their contribution to DM in the reference diet; indicated by the term *VEM-intake maize silage*), and standard concentrates for grass herbage, grass silage and maize silage (the latter three in proportion to their contribution to DM in the reference diet; indicated by the term *VEM-intake concentrates*).

3.2.3 Chemical composition of dietary components

Uncertainty not only involves that of DM-intake or of the contribution to total VEM-intake by a certain dietary component. Analysis of the chemical composition also adds an uncertainty. The size of this uncertainty was deduced from the variation in analysed chemical composition since the year 1990 (see Appendix 2). Yearly variation in chemical composition was hence taken as a measure for uncertainty. Similar to the calculations in Section 3.2.2 an equal VEM-intake by the cow was assumed.

Different chemical fractions in a dietary component were exchanged based on their contribution to DM (to ensure 100% coverage of DM). In case these changes in chemical composition were thought to be associated with a changed VEM-value of that dietary component (derived from values reported in feeding tables), DM-intake was of the total diet was corrected to ensure that VEM-intake remained equal and comparable to that of the calculations in Section 3.2.2. For grass herbage, grass silage and maize silage these corrections were performed in combination with changes in sugar content, sugar content and starch content, respectively.

In the other cases it was assumed that a change in chemical composition did not affect the VEM-value of dietary DM, the VEM-intake by the cow and the VEM-requirement to achieve the milk production level that has been registered.

The effect of uncertainty about chemical composition was tested by exchange of individual chemical fractions against each other on the basis of DM. It was presumed that there is a relatively higher uncertainty of the sugar content than of the protein content in grass herbage and grass silage, and that with maize silage uncertainty of starch content is most relevant. There are different causes of these uncertainties, such as samples of grass and maize silages that differ from average composition of these silages as actually fed on Dutch dairy farms, a moment of sampling that is not representative of the average silage composition, and material offered for analysis that is not representative of the average Dutch dairy farms. The choice was made to make the VEM-value of grass herbage and grass silage dependant only on crude protein content, and that of maize silage only on starch content. The historic series of analysis values for grass herbage, grass silage and maize silage were consulted here, instead of the nutritional consequences of changes in chemical composition. The changes in VEM-value were hence not derived with arguments concerning feed digestibility (energy value attributed to the digestion of sugars, protein and starch).

The sugar content in grass herbage and grass silage was exchanged by cell wall material by 20 g of sugar/kg DM[1] (indicated by the term *sugar grass herbage* and *sugar grass silage*, respectively). First, the exchange of analysed sugar and cell wall content was performed, followed by allocation of the fraction not accounted for by all analysed chemical fractions equally to both the sugar and the cell wall fraction. This exchange was also tested in combination with a change of the crude protein (CP) content of 10 g CP/kg DM[1] (indicated by the term *sugar&CP grass herbage* and *sugar&CP grass silage*) where the change in CP content was first subtracted from the cell wall fraction, and subsequently performing the exchange between the sugar and cell wall fraction as described above. For the combination with a change of CP content also the VEM-value of grass herbage and grass silage was assumed, based on historical measurements (see Appendix 2) and available tables of feed values.

The VEM value of grass herbage of 957 VEM/kg DM for the reference situation increased to 1000 VEM with a CP content of 230 g CP/kg DM and decreased to 945 VEM with a CP content of 170 g CP/kg DM.

The reason for the relative small decrease of the VEM value is that the CVB-feeding tables (CVB, 2007) indicate for grass harvested at a late stage of maturity and a CP content of 172 g CP/kg DM a VEM value of 984 VEM/kg DM. For this reason a choice was made to limit the decrease of VEM value to a small size.

The VEM value of grass silage increased from 891 VEM/kg DM for the reference situation to 895 VEM / kg DM with a CP content of 180 g CP/kg DM and decreased to 860 VEM / kg DM with a CP content of 150 g CP/kg DM[1]. These estimates were derived from the changes in VEM value associated with a changed CP content in grass herbage and grass silage as mentioned in the CVB-feeding tables (CVB, 2007). The aim was to include the general effects of CP content on feed intake level. The changes in VEM values were not based on nutritional evaluation and the contribution of the separate chemical fractions to the VEM value. The evaluation of such nutritional backgrounds would require a far more detailed study.

An uncertainty of 25 g starch/kg DM[2] in maize silage was investigated by exchanging this fraction with the fraction of cell walls (indicated by the term *starch maize silage*). Comparable to the calculation for grass, the exchange between starch and cell walls was performed on the contents in maize silage analysed. Subsequently, a rest which was not allocated to any of the chemical fractions analysed was divided equally over the starch and cell wall fraction. Also in this case a change of VEM value was assumed based on reported differences in VEM value with a change in starch content in CVB-feeding tables (CVB, 2007). The VEM value of maize silage of 977 VEM/kg DM increased to 995 VEM/kg DM with a starch content of 400 g starch/kg DM and decreased to 950 VEM with a starch content of 300 g starch/kg DM [2] (see Appendix 2; table values).

Finally, an uncertainty of 50 g starch/kg DM in standard concentrates was investigated (indicated by the term *starch concentrates*). As a result of the strong variation in the composition of concentrates offered in the dairy sector, of the strongly varying starch content and of the uncertainty about the characteristics of individual deliveries which are used to produce compound feed for dairy cows, a higher uncertainty was assumed than the variation in starch in maize silages according to historic data. Starch was exchanged with sugars (indicated by the term *starch/sugars concentrates*) or cell walls (indicated by the term *starch/cell walls concentrates*). Although to a lesser extent, there is also variation and uncertainty with respect to CP content, and CP was exchanged with sugars and starch in proportion to their content in DM. The effect was tested of in-crease and decrease of CP with 25 g CP/kg DM (indicated by the term *CP/sugar&starch concentrates*).

3.3 Uncertainty of model representation

Besides uncertainty around model input values, also an inappropriate representation of the microbial fermentation processes (which determine the amount and type of VFA formed, the hydrogen surplus and formation of CH_4 from this hydrogen) contributes to uncertainty of predicted CH_4 production. For an impression of the extent to which the model representation contributes to this uncertainty, the influence was investigated of some important internal model parameters and some internal calculation rules.

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[1] For grass herbage as well as grass silage an uncertainty of the analysis (representing the average conditions in the Netherlands) of sugars of 20 g sugars/kg DM and 10 g CP/kg DM was considered realistic. It was assumed that the historic values of analysed sugar content are less representative to derive an average value of sugar content in truly consumed grass herbage and grass silage on Dutch dairy farms than those of crude protein content. This implies that, in contrast to the deviation of the VEM-value, the variation of historic analysis results of sugar and crude protein content were not leading for estimates of these uncertainties.
The effect of these uncertainties was calculated by interpolation of the simulation results obtained with extreme values for sugars (60 and 120 g sugars, and 170 and 230 g CP/kg DM of grass herbage; 70 and 130 g sugars, and 150 and 180 g CP / kg DM of grass silage).

[2] An uncertainty of 25 g starch/kg DM was considered realistic. The effect of this uncertainty was calculated by interpolation of the simulation results obtained with extreme starch contents of 300 and 400 g starch/kg DM.

3.3.1 Model parameters

The model parameters for the rather physical aspects of rumen function strongly determine the model outcomes as demonstrated by sensitivity analysis of Neal *et al.* (1992) and Bannink *et al.*, (1997c). The influence of uncertainty around the fractional rates of passage of particulate material was evaluated by a increase or a decrease by 0.1 /d (indicated by the term *particle passage*). This accounts for a deviation of about 10% of the value for the reference situation. A larger uncertainty of 0.2 /d was adopted for the fractional rate of fluid passage because this fractional rate has a much higher value than that for particulate material (indicated by the term *fluid passage*). Finally, also uncertainty around rumen fluid volume was evaluated by adopting a 10 litre increase or decrease of this volume (indicated by the term *rumen volume*) which accounts for more than 10% deviation of the reference value.

Besides these physical aspects, also chemical aspects of rumen fermentation conditions are dictating the course of rumen fermentation. In particular changes in rumen acidity are strongly associated to the effects of altered rumen fermentation conditions on microbial activity (Dijkstra *et al.*, 1992), rate of VFA production, hydrogen balance and CH_4 production rate (Bannink *et al.*, 2005). Uncertainty round of rumen acidity was evaluated by a deviation by 0.1 pH unit from the reference situation (indicated by the term *acidity*). With this alteration of the daily average of rumen pH also the minimum pH and the time period with pH lower than 6.3 were estimated according to the empirical equations of Mills *et al.* (2001).

The assumption that no changes occurs in feed intake with changes of the above mentioned model parameters is debatable. An increase of fractional passage rates means a smaller retention time in the rumen, and hence less time for micro-organisms to ferment the feed. As a result, de VEM value will be lower when this concerns cell wall material, whereas the VEM value actually may increase when more starch and protein escape rumen fermentation and is digested in the small intestine. Therefore, an evaluation of the effects of passage, which includes changes in VEM value, must always be performed for a specific diet. With the calculations performed in the present study, an upward or downward corrections of the VEM values was not taken into account.

3.3.2 Internal calculation rules

Uncertainty around some important internal calculation rules is of particular importance for calculations of CH_4 production. On the first place, this involves the representation of VFA production from fermented substrates. A higher proportional production rate of propionic acid at the expense of acetic acid and butyric acid (per unit of fermented substrate converted into VFA) results in a smaller hydrogen surplus and hence less CH_4 produced. Predicted CH_4 production is therefore mainly the complement of predictions of amount and type of VFA produced, and is sensitive for the model representation of the so-called stochiometry of VFA yield from fermented substrates. Current insight from available research results indicates that the model version currently used in the Dutch protocol delivers the best estimates of VFA production (Bannink & Tamminga, 2005; Bannink, 2007; Bannink *et al.*, 2008; Dijkstra *et al.*, 2008a).

With the aim to obtain an impression of the sensitivity of predicted CH_4 production rate for the internal calculation rules for VFA production and the uncertainty associated with them, some alternative representations of the stoichiometry of VFA yield from fermented substrates were compared. This comparison involved the following three alternative representations of VFA production on Dutch dairy rations rich in forage:

1) A representation according to results from Bannink & Dijkstra (2005) and applied by Bannink *et al.* (2005, 2008 & 2010). This representation is applied in the current Tier 3 approach for the Dutch ER protocol. The representation was derived from exclusively in vivo observations in the rumen of high-yielding dairy cows and represents the effect of rumen acidity on stoichiometry of VFA yield from fermented substrates (indicated by the term *VFA-stoichiometry, Bannink 2005*)
2) A representation according to results from Bannink *et al.* (2000, 2006) and applied by Mills *et al.* (2001). This representation was derived from the same data set as 1). However, the effect of rumen acidity on VFA production with fermentation of sugars and starch is lacking in this representation of stoichiometry of VFA yield from fermented substrate (indicated by the term *VVZ-stoichiometry, Bannink 2000*).
3) A representation according to the results of Murphy *et al.* (1982) and applied by Dijkstra *et al.* (1992). This representation was derived to a large extent from other ruminants than high-yielding, lactating dairy cattle (indicated by the term *VVZ-stoichiometry, Murphy 1982*). It is known for quite some time that this representation is inappropriate for high-yielding dairy cows (Neal *et al.*, 1992; Bannink *et al.*, 1997a, 1997b).

A comparison was made between these three alternatives by performing calculations which differed only in the representation of stoichiometry of VFA yield from fermented substrates,

Besides the rumen production CH_4, to a much smaller extent also CH_4 production in the large intestine contributes to total CH_4 emission in cows. Large intestinal fermentation contributes less than 10% of total enteric CH_4 production in dairy cows, and hence can certainly not be neglected. It was tested to what extent the large intestine contributes in the reference situation and what error is to be expected when neglecting this contribution (indicated by the term *no large intestine*). In addition, uncertainty around the contribution of the large intestine was tested by increasing and decreasing the fractional passage rate of large intestinal contents by 50% (indicated by the term *passage large intestine*).

3.3.3 Rumen fat metabolism

Rumen fat metabolism affects CH_4 production in various ways. In addition to the error analysis in Section 3.3.1 and Section 3.3.2 a sensitivity analysis was performed for effects of manipulations in dietary fat on predicted CH_4 production. The error made with assumption on the fraction of crude fat in dietary dry matter is probably small, because the fraction of crude fat is always of a much smaller size than that of sugars, starch, cell wall material (analysed as NDF for Neutral Detergent Fibre) and protein. The contribution to uncertainty of the predicted MEF and MCF values for the average Dutch dairy cow hence remains small as well. In case it will be common practice to manipulate dietary fat content and fat composition (improved milk fat quality, or aiming at reduction of CH_4) this aspect does need to be taken into account when estimating uncertainty of MEF and MCF predictions.

A high dietary fat content reduces cell wall degradation rate, whereas a high concentration of solubilised unsaturated long chain fatty acids inhibit the activity of micro-organisms degrading cell wall material and of protozoa (Dijkstra *et al.*, 2000). Acidity of rumen fluid affects the rate of hydrolysis of fat to solubilised long chain fatty acids, and affects the rate of hydrogenation (saturation) of unsaturated long chain fatty acids to saturated long chain fatty acids (Bannink & Dijkstra, 2006). The representation of these processes and of the interaction between fat, long chain fatty acids, the degree of saturation of long chain fatty acids, rumen acidity and microbial activity are not included in the model used for the current Dutch ER protocol. These model elements hence had to added to the model. Besides representation of the effect of nutrition on rumen fermentation processes in the original mode, in the extended model also the effects of fat and fat composition no this fermentation were included. After this extension of the model rumen acidity not only has a direct impact on the activity of cell wall degrading micro-organisms. It also affects the extent of fat hydrolysis and of hydrogenation of unsaturated long chain fatty acids, and the rumen concentration of unsaturated long chain fatty acids and its direct impact on cell wall degradation (Dijkstra *et al.*, 2000).

Because of extension of the model with fat metabolism, additional model inputs were needed with respect to the dietary content of saturated and unsaturated free long chain fatty acids (in the Tier 3 for the current Dutch ER only a fat fraction is defined), and to the proportion of unsaturated and saturated fatty acids in dietary fat. The fat content already was a model input in the original model. For the reference situation holds that concentrates contain 5% fat (50 g fat / kg DM). For calculations with the representation of rumen fat metabolism in the reference situation it was assumed that fat is composed of 20% unsaturated and 80% saturated long chain fatty acids. The effects of variation in fat content and fat composition in comparison to that in the reference situation was tested in three alternative ways:

1) By varying the fat content of concentrates (indicated by the term *fat content*) with an exchange of fat by rapidly fermentable carbohydrates (sugars and starch were in proportion to their contribution to dietary DM. A fat content of 5% for the reference situation was decreased to 2% or increased to 10% and 15% of DM in concentrates. Because the VEM value of fat in g/kg DM is about three times as higher than for sugars and starch, DM intake of concentrates was changed to maintain an equal VEM intake with concentrates as for the reference situation.
2) By varying the degree of saturation of fatty acids in non-hydrolysed fat (indicated by the term *fat saturation*). The degree of saturation of fatty acids in non-hydrolysed fat was varied from 20% to 80%. This saturation applies for the total fraction of crude fat in the ration. Two dietary fat contents were evaluated: a content equal to that in the reference situation, and a highest content of 150 g of crude fat/kg DM of concentrates.
3) By varying rumen acidity from 5.5 to 6.5, in combination with a variable fat content (indicated by the term *acidity & fat content*) and variable degree of saturation of long chain fatty acids in fat (indicated by the term *acidity & fat saturation*)**.** Besides the effect of acidity on fat hydrolysis and on the hydrogenation of unsaturated long chain fatty acids, acidity also affects cell wall degradation. Both effects are exerted simultaneously when predicting MEF and MCF when altered rumen acidity is imposed on the model (in contrast to prediction of pH values by the model itself; Table 1B).

3.4 Results & discussion: uncertainty of model inputs

Calculated changes in MEF (in kg CH_4/cow/year) and in MCF (in % of GE-intake) are given in Table 4. Figures 3 to 8 show the same results, but expressed as the percentage of change compared to estimate MEF and MCF value for the reference situation. In Table 5 these percentages of change for all uncertainties investigated were summed and converted into an estimate of the total uncertainty of MEF and MCF predictions by the model used as a Tier 3 approach in the Dutch ER protocol for CH_4 emission in dairy cows.

3.4.1 Feed intake

Feed losses

Correction for feed losses results in a 3.8% reduction of DM intake and a 2.6% reduction of the MEF value (in kg CH_4/cow/year; Table 4 and Figure 3) compared to not correcting for such losses (the reference situation). The value of MCF (in % of GE-intake) increased 0.3%. The revised methodology for the national inventory of diet and performance of dairy cows includes this correction for feed losses. Hence, only the size of these losses is an uncertainty which makes the present result an exaggeration it of uncertainty related with assumptions on feed losses.

DM intake ration

An increase or a decrease of total DM intake of 2% compared to the reference situation with equal dietary composition (corresponding to a 2% increase and decrease, respectively of VEM-intake) resulted in a 1.6% increase or decrease of the MEF value, respectively. The MCF value changed by -0.3% and +0.5% (Table 4 and Figure 3).

DM intake grass herbage

An increase or decrease of grass herbage intake equal to 10% of the sum of DM intake of all grass products in the reference situation (DM intake non-grass products remained equal) resulted in a 5.2% increase or decrease of total DM intake. This caused a 4.5% higher or lower MEF values, respectively, whereas the MCF value decreased with 0.5% or increased with 0.7%, respectively (Table 4 and Figure 3).

The 4.5% change of MEF is large compared to the 5.2% change in DM intake and indicates that compared to the other dietary components relatively much CH_4 is emitted from grass herbage. Despite the relatively high contribution of grass herbage to MEF, the MCF decreased still as a result of the higher DM intake.

DM intake grass silage

An increase or a decrease of grass silage intake equal to 10% of the sum of DM intake of all grass products in the reference situation (DM intake non-grass products remained equal) resulted in a 5.2% increase or decrease of DM intake and a 4.0% higher or a 4.1% lower MEF value, respectively. This was accompanied by a 1.0% decrease or a 1.2% increase of the MCF value.

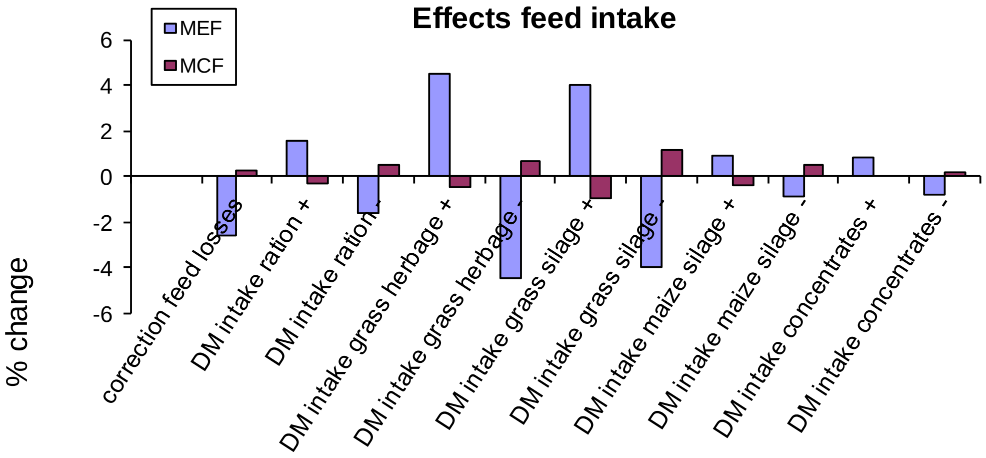

Figure 3. Overview of the effect of an increase (+) or decrease (-) in the total DM intake or DM intake of specific dietary components. Effects are expressed as changes (in %) in MEF and MCF compared to the values calculated for the reference situation.

Table 4. Effects of changes in model input, in some internal model parameters, and in some internal calculation rules used in the model, expressed as absolute values of the methane emission factor (kg methane/cow/year; MEF) and as methane as a percentage of gross energy (GE) intake (MCF).

		MEF **kg CH_4/cow/yr**	**MCF** **(% GE intake)**
Reference (current ER)		129.4	5.91
Term used for change with respect to reference	**Type & size of change**		
Feed intake (unequal DM and VEM intake)			
Feed losses (including correction for feed losses)	- 3.8% DM intake, according to WUM	126.1	5.92
DM intake (equal dietary composition)	+ 2% DM intake	131.4	5.89
DM intake grass herbage (unequal dietary composition)	+ 10% of DM intake of all grass products (+5.2% total DM intake)	135.2	5.88
DM intake grass silage (unequal dietary composition)	+ 10% of DM intake of all grass products (+5.2% total DM intake)	134.6	5.85
DM intake maize silage (unequal dietary composition)	+ 5% of DM intake of maize silage (+1.3% total DM intake)	130.6	5.88
DM intake concentrates	+ 5% of DM intake of concentrates (+1.0% total DM intake)	130.4	5.90
Partition individual feed components (equal VEM intake)			
VEM intake grass herbage (exchange grass & maize silage, conc.)	+ 10% total DM intake	130.6	5.97
VEM intake grass herbage/grass silage (exchange grass silage)	+ 10% total DM intake	129.9	5.97
VEM intake maize silage (exchange grass herbage & silage)	+ 10% total DM intake	127.5	5.84
VEM intake conc. (exchange grass herbage, grass & maize silage)	+ 10% total DM intake	128.4	5.94
Composition dietary components [1]			
Sugar grass herbage (exchange cell walls)	+20 g sugar/kg DM grass herbage	129.5	5.92
Sugar&CP grass herbage (exchange cell walls)	- 10 g CP/kg DM grass herbage	129.5	5.92
Sugar grass silage (exchange cell walls)	+20 g sugar /kg DM grass silage	130.1	5.94
Sugar&CP grass silage (exchange cell walls)	- 10 g CP/kg DM grass silage	131.4	5.95
Starch maize silage (exchange cell walls)	+25 g starch/kg DM maize silage	129.6	5.93
Starch/sugar concentrates (exchange sugar)	+50 g starch/kg DM conc.	128.8	5.88
Starch/cell walls concentrates (exchange cell walls)	+50 g starch/kg DM conc.	129.3	5.91
CP/sugar&starch concentrates (exchange sugar, CP)	+10 g CP/kg DM conc.	129.3	5.90

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Model parameters [1]			
Particle passage	+0.1 /day	127.7	5.83
Particle passage	- 0.1 /day	130.4	5.96
Fluid passage	+0.2 /day	128.5	5.87
Fluid passage	- 0.2 /day	130.1	5.94
Rumen volume	+10 litre	130.7	5.97
Rumen volume	- 10 litre	128.0	5.85
Acidity	+0.1 pH unit	123.8	5.65
Acidity	- 0.1 pH unit	131.9	6.02
Internal calculation rules			
VVZ-stoichiometry Bannink 2000	alternative stoichiometry forage-rich diets	142.0	6.49
VVZ-stoichiometry Murphy 1982	alternative stoichiometry forage-rich diets	153.1	6.99
No large intestine	neglecting of large intestine	117.9	5.39
Passage large intestine [1]	+50% change	129.0	5.89
Passage large intestine [1]	-50% change	129.8	5.93
Fat content [1]	fat content +15g/kg DS q	128.0	5.87
Fat saturation [1]	degree of saturation fatty acids +5%	129.4	5.91
Acidity & fat [1]	+0.1 pH unit in combination with fat	131.2	5.91
Acidity & fat [1]	-0.1 pH unit in combination with fat	123.2	5.87

[1] *These results were calculated by interpolation of results for a wider range of values tested (see Chapter 4 for a description of this range). The change in values mentioned in Table 4 is the inaccuracy thought to be realistic for these values.*

The decrease of MEF of 4% is of a smaller size than calculated for grass herbage but remained relatively large. This indicates that also the contribution of grass silage to CH_4 is relatively large. Again, the MCF value decreased with an increase of DM intake.

DM intake maize silage

An increase or decrease of maize silage intake equal to 5% of total DM intake in the reference situation (intake of non-maize dietary components kept equal) resulted in a 1.3% increase or decrease of total DM intake respectively, which was accompanied by a 0.9% higher or lower MEF value, respectively. The MCF value decreased with 0.4% or increased with 0.5%, respectively.

The effect of a higher intake of maize silage on MEF was about half the effect of a higher intake of grass silage. This indicates the contribution of maize silage to CH_4 is relatively low compared to the other dietary components. Also the decrease of MCF with an increased intake of maize silage was about half of that with an increased intake of grass herbage or of grass silage.

DM intake concentrates

An increase or decrease of concentrates intake equal to 5% of total DM intake in the reference situation (intake of all other dietary components kept equal) corresponds to about 1% increase4 or decrease of total DM-intake, respectively. These changes caused a 0.8% increase or decrease of MEF, respectively, accompanied by a 0.0% decrease or 0.2% increase of MCF, respectively.
The effect of higher intake of concentrates on MEF is less than half of the effect of higher intake of grass herbage or grass silage. The effect is also 10% smaller that the effect of a higher intake of maize silage. These results indicate that concentrates contributes least to CH_4 from all dietary components tested. With an increase of DM intake the MCF value hardly decreased, whereas it did with a decreased intake of concentrates. This MCF increase was about half in size of that with a decreased intake of maize silage, and a third of that with a decrease intake of grass silage.

In conclusion

The results presented here indicate that an increase of feed intake is accompanied with an increase of MEF and a decrease of MCF as a results of the effects of higher feed intake on rumen fermentation processes (such as rumen acidity and passage rates). The extent of change of MEF and MCF depends on how higher fed intake is achieved. Estimated intakes of grass herbage and grass silage are to be most uncertain, besides estimates of feed losses and estimated feed requirement according to the VEM-system. Error introduced with these three estimates leads to a total uncertainty of 5% for MEF and 1.5% for MCF. This uncertainty is much lower than the summed total of all above mentioned results because the assumption of independency of uncertainty of DM intake with grass herbage, grass silage, maize silage and standard concentrates (Figure 3) is not realistic. They must be considered dependant because a higher or lower intake of some dietary component will be compensated by a lower and higher intake, respectively, of the other dietary components. Summing of all uncertainties calculated would hence be a strong overestimation of the effect of uncertainty around DM or VEM intake on uncertainty of the MEF and MCF values predicted by the model.

Most determinant for MEF and MCF uncertainty is the effect of estimated milk production level in the reference situation, and the effect of the DM and VEM intake required to achieve this milk production (feed losses, DM intake ration). Uncertainty of VEM intake because of inaccurate estimates of milk production level was estimated by the WUM to be 2%. This error estimate corresponds with about 0.5 kg milk/cow/day and with 2.5% of daily VEM intake. With an additional 2% uncertainty of daily VEM intake and of feed losses, an uncertainty of 5% with respect to DM intake seems to be realistic for the average Dutch dairy cow. This corresponds to about 4% uncertainty of MEF predictions and 1% of MCF predictions. Compared to these uncertainties, the effect of an inappropriate correction for feed losses seems to be considerable (2.6% and 0.3% respectively).

Attributing such a 5% uncertainty of DM intake fully to intake of grass herbage leads to uncertainty of predicted MEF of 4.5%. Attributing this uncertainty to grass silage intake instead of grass herbage results in a 11% lower uncertainty of predicted MEF. When attributing this uncertainty to intake of maize silage or standard concentrates a 20% or 8% lower uncertainty is obtained, respectively (a 80% larger, 25% smaller and 60% smaller uncertainty of MCF was established for grass silage, maize silage and concentrates, respectively).

It is concluded that the uncertainty of estimates of VEM or DM intake probably have to be attributed most to uncertainty of estimated intake of grass products. This uncertainty is responsible for a 4.5% uncertainty of the predicted MEF value (and 1% of predicted MCF value). The effect of uncertainty of DM intake is hence far more important than that of registered milk production. Because an inaccurate estimation of milk production per cow is always independent from uncertainties around dietary composition and estimated DM and VEM intake, uncertainties associated with both aspects needs to be added. Summation of both leads to an estimate of uncertainty of 5% of predicted MEF and of 1.5% of predicted MCF.

3.4.2 Partition dietary components

VEM intake grass herbage

Uncertainty (increase or decrease) with respect to intake of grass herbage equal to 10% of total DM intake (VEM intake grass herbage exchanged with VEM intake with grass silage, maize silage and concentrates in proportion to their contribution to dietary DM) resulted in +0.9% and -0.9% change of predicted MEF value, respectively. The accompanying change in predicted MCF value was +1.1% and -1.0%, respectively.

VEM intake grass herbage / grass silage

An uncertainty (increase or decrease) with respect to the intake of grass herbage equal to 10% of total DM intake (VEM intake of grass herbage exchanged with VEM intake of grass silage only) resulted in a change of MEF value of just +0.3% and -0.3%, respectively. This was accompanied by +1.0% or -0.9% change of predicted MCF, respectively.

VEM intake maize silage

Uncertainty (increase or decrease) with respect to the intake of maize silage equal to 5% of Total DM intake (VEM intake maize silage exchanged with VEM intake grass herbage and grass silage in proportion to their contribution to dietary DM) resulted in -0.8% and +0.7% change of predicted MEF, and -0.7% and +0.7% change of MCF, respectively.

VEM intake concentrates

Uncertainty (increase or decrease) with respect to the intake of standard concentrates equal to 5% of total DM intake (VEM intake concentrates exchanged with VEM intake grass herbage, grass silage and maize silage in proportion to their contribution to dietary DM) caused a -0.4% and +0.4% change of MEF and a +0.2% or -0.2% change of MCF, respectively.

In conclusion

These uncertainties of predicted MEF and MCF values with a changed partition of VEM-intake over grass herbage, grass silage, maize silage and standard concentrates can not be summated (Figure 4). Assuming an equal VEM intake, the combination of uncertainties with respect to the proportion of dietary components in dietary DM will cause an uncertainty which lies between the minimum and maximum of the values established. It is plausible that uncertainty with respect to grass intake is most important and this will hence contribute strongly to uncertainty around the chemical composition of dietary DM and of predicted MEF and MCF values. It is concluded that the partition of the required VEM intake over the various dietary components contributes to 1% uncertainty of MEF as well as of MCF.

3.4.3 Composition of dietary components

The chemical fractions in a specific dietary component were exchanged on a DM basis (100% coverage of component DM).

Sugar grass herbage; sugar&CP grass herbage

A increase or decrease of the sugar content in grass herbage of 20 g sugar/kg DM (exchange with cell walls; NDF) resulted in +0.1% or -0.1% change MEF-value, respectively. In combination with an uncertainty of the CP-content of 10 g CP/kg DM these changes increased in size to +0.2% or -0.2%, respectively. The MCF value changed with +0.2% or -0.2%, respectively, with or without a change of CP content.

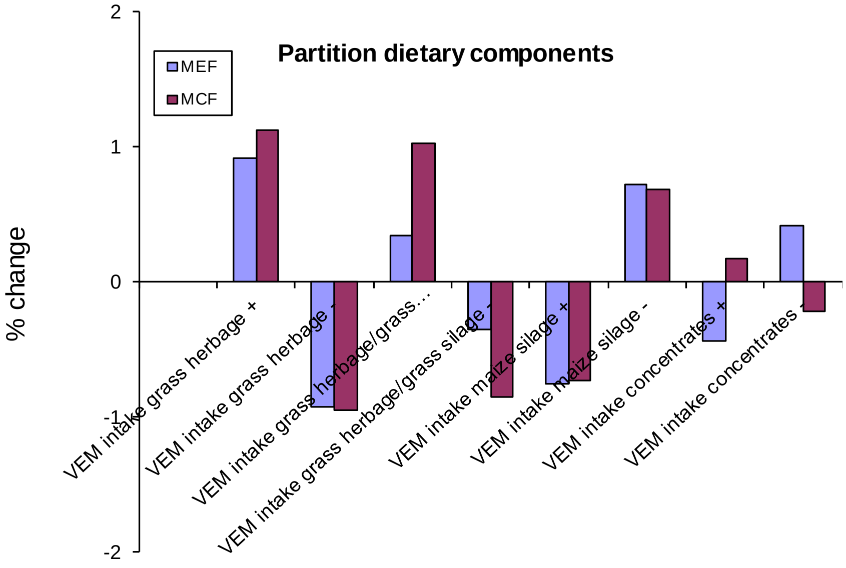

Figure 4. Overview of the effect of an increase (+) or decrease (-) in the proportion of a specific dietary component of dietary DM. Effects are expressed as changes (in %) in the MEF and MCF values compared to the values calculated for the reference situation.

Sugar grass silage; sugar&CP grass silage

An increase or decrease of the sugar content in grass silage of 20 g sugar/kg DM (exchange with cell walls; NDF) resulted in a +0.5% or -0.5% change of MEF value. In combination with an uncertainty of the CP-content of 10 g RE /kg DM this change increased in size to +1.0% or -1.0%, respectively. The MCF value changed with the same sign with +0.5% or -0.5%, respectively, and with +0.7% or -0.7%, respectively when in combination with change in CP content.

Starch maize silage

An increase or decrease of the starch content of maize silage of 25 g starch /kg DM (exchange with cell walls; NDF) resulted in a change of -0.04% or +0.04% of the MEF value, respectively. This was accompanied by a change of +0.4% or -0.4% of the MCF value, respectively.

Starch/sugar concentrates; starch/cell wall concentrates

An increase or decrease of the starch content of standard concentrates of 50 g starch /kg DM, in exchange with sugars, resulted in a change of MEF value of -0.5% or +0.5%, respectively. When in exchange with cell walls (NDF) this change was of a much smaller size of -0.1% en +0.1%, respectively.

Increase or decrease of starch content in exchange with sugars was accompanied by a change of MCF value of -0.5% or +0.5%, respectively, but when in exchange with cell walls this change reduced in size to +0.01% or -0.01%, respectively.

CP/sugar&starch concentrates

An increase or decrease of the CP content in standard concentrate of 10 g CP/kg DM, in exchange with sugar and starch in equal proportion to their content in dietary DM, in a change of MEF value of -0.1% or +0.1%, respectively. The change in MCF value was -0.2% or +0.2%, respectively.

In conclusion

The calculated effects of uncertainties with respect to chemical composition of the most important dietary components on uncertainty of MEF and MCF are to a large extent independent. They will mainly depend on errors made with 'sampling' of these components (e.g., samples not representative, analysis results do not reflect the average Dutch circumstances because not all silages have been analysed) and errors with analysis of these samples (forages are estimated based on calibration lines derived for the NIRS method, instead of actual chemical analysis; for other dietary components estimates also based on table values and statistics of feed utilization by the dairy sector). Because of the independence of the type of error made with the above mentioned errors, the associated uncertainties calculated for MEF and MCF may be summed. This means that uncertainty as a result of uncertainty with respect to the chemical composition of dietary components is in the order of 2% for both MEF and MCF (Figure 5).

With estimation of the uncertainty it was in some cases assumed the VEM value of the dietary component remained equal despite a change in chemical composition. This will often not be the case however and for this reason the change in chemical composition will be confounded with a change in VEM value of that component and the DM intake required to cover VEM–requirement. This confounding is difficult to evaluate and can only be studied with simulations aimed at specific production conditions. A nutritional analysis to such a detailed level was not the aim of the present study however.

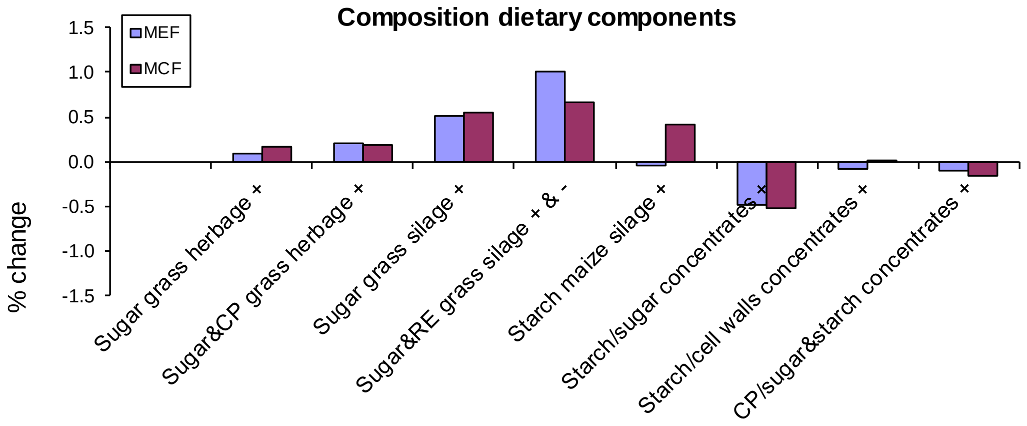

Figure 5. Overview of the effect of an increase (+) or decrease (-) in a chemical fraction of a dietary component. Effects are expressed as changes (in %) in the predicted MEF and MCF values compared to the values calculated for the reference situation. The values shown are an interpolation of the values predicted for extreme sugar, starch and CP content in grass herbage, grass silage and maize silage.
With a change in CP content of grass herbage and grass silage, and with a change in starch content of maize silage also the VEM values was assumed to change, and total dietary DM intake had to be corrected to achieve equal VEM intake to that in the reference situation.

3.5 Results & discussion: uncertainty of model representation

The following sections describe the results for the effects of uncertainty in internal model parameters and in internal calculation rules on the uncertainty of predicted MEF and MCF values.

3.5.1 Model parameters

Particle passage
An increase of the fractional passage rate of particulate matter (the daily rate at which particulate matter present in the rumen is being replaced) with 0.1 /d resulted in a 1.1% decreased of predicted MEF, and an identical 1.1% decrease of predicted MCF.

Fluid passage
An increase of the fractional [passage rate of fluid with 0.2 /d resulted in a 0.6% decrease of predicted MEF and MCF.

Rumen volume
An increase in rumen fluid volume with 10 litres resulted in a 1.0% decrease of predicted MEF and MCF.

Acidity

An decrease or increase of the acidity of rumen contents (a increase or decrease of pH, respectively) with 0.1 pH unit resulted in a +1.9% and -4.3% change of both MEF and MCF, respectively. This result indicates that an increased acidity (decrease of pH) has a much stronger effect on MEF and MCF than a decrease of acidity (increase of pH). Uncertainty with respect to rumen acidity strongly contributes to uncertainty of predict MEF and MCF values.

In conclusion

The uncertainties calculated in this section are rather independent from each other. Together they contribute to the uncertainty of predicted MEF and MCF values in the order of 6% for both (Figure 6). This result indicates that predicted CH_4 emission is highly sensitive to the assumptions made for the values of these model parameters. The sensitivity calculated in the present study is however not a direct measure for the uncertainty introduced when applying MEF and MCF for CH_4 calculations. Effects on the ration formulation, on rumen digestion, on DM intake and on milk production level achieved were not taken into account in the present study. It was inherently assumed these factors remained the same despite the changes introduced for rumen acidity and passage rates. This will in general not be the case however (Dijkstra *et al.*, 1992).

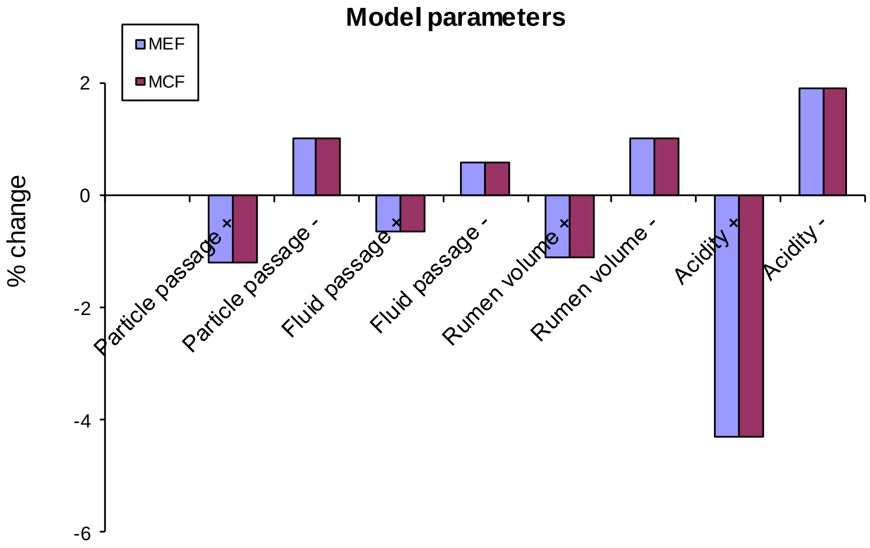

Figure 6. Overview of results for the effect of an increase (+) or decrease (-) in some important internal model parameters. Effects are expressed as changes (in %) in the predicted MEF and MCF values compared to the values predicted for the reference situation.

A higher passage rate or a reduced rumen pH normally leads to a lower feed digestibility and also a changed stoichiometry of VFA-production in the rumen. This indeed yields less CH_4 but it may also be accompanied by a lower feed energy value of the feed if the diet has a high cell wall content, but an increased feed energy value if the diet is rich in protein or starch. An average Dutch diet for dairy cows normally contains a higher cell wall fraction than that of starch plus protein, and hence a decrease of the feed energy value is plausible with increase of passage rates and rumen acidity. In case the feed energy value decreases the dairy cow would need to consume more DM to achieve the same milk production level. A higher DM intake stimulates rumen fermentation however and CH_4 production. This stimulatory effect on CH_4 hence compensates at least part of the simulated decline of CH_4 as a result of higher passage rate and rumen acidity. An uncertainty of DM intake of 5% of the reference diet already causes a 3% increase of predicted MEF (see Section 3.4.1). For this reason, it seem realistic to let the uncertainty with respect to internal model parameters account for a 3% uncertainty in predicted MEF values at maximum. The contribution to the uncertainty of MCF probably remains higher because MCF is four times less sensitive to changes in DM intake with equal dietary composition (see Section 3.4.1) An uncertainty of 5% is thought to be realistic for MCF.

Uncertainty caused by inaccuracy of the value of some important internal model parameters clearly cannot be summated, and they cannot be taken into account for 100% with respect to their contribution to uncertainty of predicted MEF and MCF. This would lead to a strongly overestimated uncertainty. Adapting these model parameters is not independent from the calculations performed in Sections 3.4.1, 3.4.2 and 3.4.3. It is quite a challenge to investigate the precise relationships and the dependencies between them, which is also strongly dependent on the type of ratio (ratio of forages to concentrates, type of forages, type of concentrates, DM intake level, and so on). The investigation of these relationships asks for highly specific studies of the subject, such as for example the recent study of Bannink *et al.* (2009) of the effect of grass quality on CH_4 production at various levels of DM intake.

3.5.2 Internal calculation rules

Production of volatile fatty acids

According to the Tier 3 approach, as described in the Dutch ER-protocol (Van der Maas *et al.*, 2008), and according to the reference situation of a forage-rich diet, calculations have been performed with the VFA-stoichiometry of Bannink *et al.* (2005) derived for forage-rich diets. Other choices to represent VFA-stoichiometry will change predictions of the amount and type of VFA produced, and as a consequence a changed hydrogen balance and CH_4 production.

Results of the regression study by Bannink *et al.* (2000, 2006) to derive VFA-stoichiometry were applied by Mills *et al.* (2001). In deriving these results, the variation in acidity of rumen contents, rumen volume, fractional passage rate of rumen fluid rate and fractional rate of VFA absorption was not included in the analysis (contrary to the approach adopted by Bannink *et al.*, 2005). Choice of the calculation rules for the earlier VFA-stoichiometry of Bannink *et al.* (2000, 2006) led to a 10% increase of MEF compared to the reference situation.

Even earlier results for VFA-stoichiometry of Murphy *et al.* (1982) were applied in the original rumen fermentation model of Dijkstra *et al.* (1992). These calculation rules were not exclusively based on data from lactating cows (in contrast to results of Bannink *et al.*, 2000, 2005, 2006). Moreover, a different regression model was used for analysis, which differed from the concepts adopted by Bannink *et al.* (2000, 2005, 2006) that correspond to those used in the rumen fermentation models developed by Dijkstra *et al.* (1992) and Mills *et al.* (2001). Using the VFA-stoichiometry results of Murphy *et al.* (1982) caused a 18% increase of predicted MEF.

In conclusion

The results imply that the prediction of CH_4 production is highly sensitive for the representation of VFA stoichiometry (Figure 7). The largest deviation from values calculated for the reference situation was obtained with the representation of VFA-stoichiometry for forage-rich diets of Murphy *et al.* (1982). For some time it had been suggested that this representation was inapplicable for the rumen fermentation conditions currently met in high-yielding lactating cows (Neal *et al.*, 1992; Bannink *et al.*, 1997a; Dijkstra *et al.*, 2008a). All representations of VFA-stoichiometry in ruminants that have been described in literature were extensively reviewed by Dijkstra *et al.* (2008a). The recent representation of Bannink *et al.* (2005, 2008) seems to be most applicable (Dijkstra *et al.*, 2008a) and for this reason uncertainty associated with an inappropriate representation of VFA-stoichiometry probably also reduced compared to the previous representation published in literature ((Bannink & Tamminga, 2005; Bannink *et al.*, 2008; Dijkstra *et al.*, 2008a).

A difference of 10% in predicted CH_4 production with the VFA-stoichiometry of Bannink *et al.* (2000) and that of Bannink *et al.* (2005, 2008) is remarkable. This implies that application of the former VFA-stoichiometry in the ER protocol would have led to a substantial increase of predicted CH_4 production with MEF and MCF values of 139 kg CH_4/cow/year and 6.4% of GE intake, compared to current values of 126 kg CH_4/cow/year and 5.9% of GE intake for the reference situation according to the ER protocol (Appendix 2).

It seems realistic to adopt a smaller uncertainty for MEF and MCF, as a result of uncertainty with respect VFA-stoichiometry, than the 10% mentioned above. Presuming that the current representation of Bannink *et al.* (2005, 2008) is a considerable improvement compared to earlier representations, an uncertainty of 5% is considered more realistic here.

Fermentation in large intestine

Calculations according to the Dutch ER protocol indicate for the reference situation that the large intestine contributes 9% of both the MEF and MCF value. This contribution appears small but it is too large to neglect with calculations of enteric CH_4 production.

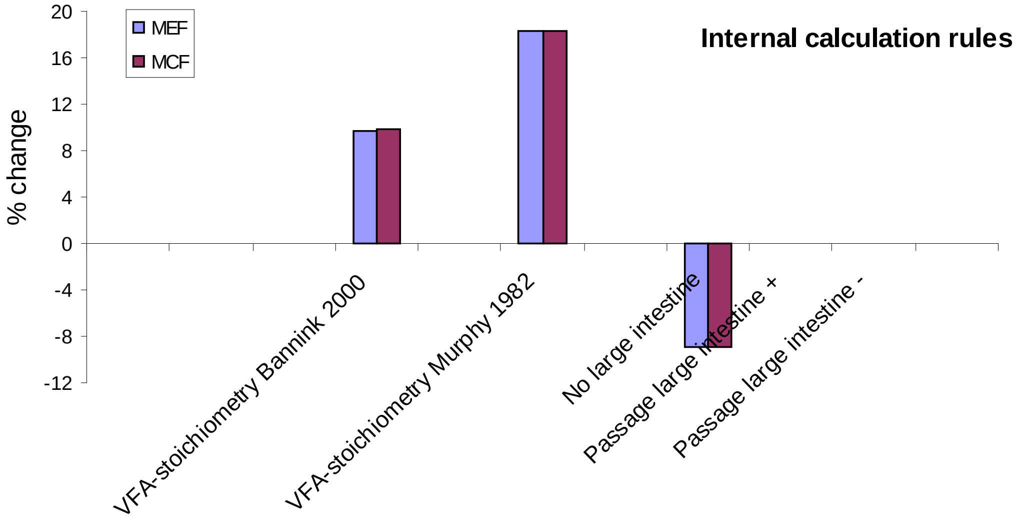

Figure 7. Overview of the effect of an alternative stoichiometry of VFA production from fermented substrates and of a change in the contribution of fermentation in the large intestine on predicted MEF and MCF values. The VFA stoichiometry according to Bannink et al. (2005) for the reference diet which is rich in forage was subsequently replaced by the VFA stoichiometry according to Bannink et al. (2000) and the VFA stoichiometry according to Murphy et al. (1982). The effect of neglecting the contribution of the large intestine to CH_4 production, and that of an altered passage rate of digesta through the large intestine were also tested. Effects are expressed as the change (in %) in the predicted MEF and MCF values compared to those calculated for the reference situation.

An increase or decrease of the fractional passage rate of the contents of the large intestine with 50% hardly affected predicted MEF. Although passage rate in principle has an effect on the retention time of particles in the large intestine, and hence also on the extent to which these particles may be fermented by micro-organisms, the calculated effect on CH_4 production for the diet of the reference situation appeared to remain very small.

In conclusion

Results of the uncertainty with respect to the contribution of the large intestine indicate that taking into account the fermentation in the large intestine which contributes slightly less than 10% of the total enteric CH_4 production is necessary. However, this contribution appeared rather insensitive to the values chosen for the model parameters related to the large intestinal compartment (Figure 7). Because the contribution of the large intestine will be sensitive to uncertainty of the dietary composition, such effects are assumed to cause a 1% uncertainty of both predicted MEF and MCF values.

3.5.3 Rumen fat metabolism

After including elements of fat metabolism in the rumen model (Dijkstra *et al.*, 2000; Bannink & Dijkstra, 2007), simulation were carried out to study the effect of dietary fat content and the degree of saturation of long chain fatty acids, either independently from each other or in combination, and both in combination with a decrease of the acidity of rumen fluid.

An increase of the fat content in concentrates (*fat content*) from 20 to 150 g/kg DM in concentrates (other assumptions with respect to fat unchanged) caused an increase of total dietary fat content from 30 to 55 g fat /kg DM. The DM intake with concentrates was corrected for the higher VEM value of fat per g DM to maintain an equal total daily VEM intake. The increase of the fat content caused a 10% decrease of MEF and a 7% decrease of the MCF. Because is not fermented in the rumen but is efficiently digested in the intestine and does deliver energy to the cow (high VEM value), the MEF and MCF are highly sensitive to the fat content of the diet. On the other hand, fat is generally a rather small fraction in dietary DM (40 g fat/kg DM for the reference situation). Uncertainty with respect to the fat content is hence also small and estimated to be less than 2.5 g fat/kg DM. This means that uncertainty of MEF and MCF due to inaccuracy of estimated dietary fat content must be 1% at maximum and less than 1%, respectively.

A theoretical change of the degree of saturation (fat saturation) of fat (or fatty acids incorporated in fat) from 20% to 80% unsaturated long chain fatty acids in fat resulted in no change of MEF and MCF with a fat content for the reference situation (50 g fat/kg concentrates DM and about 40 g fat/kg dietary DM). With an increased fat content (150 g / kg concentrates DM; 55 g fat/kg dietary DM) the same change in degree of saturation resulted in just 1% decrease of both MEF and MCF. Also cell wall degradation in the rumen decreased with an increase of the degree of fat unsaturated from 20% to 80% (0.5 %-units of digestion with the fat dietary content for the reference situation, and 2.6 %-units of digestion with the high dietary fat content). This decrease was caused by the increased rumen concentration of unsaturated long chain fatty acids in the rumen. Higher dietary fat content (which does not generate VFA and CH_4) and less fermentation of cell walls resulted in a lower production of VFA and CH_4 (predicted rumen acidity slightly decreased). The size of the effect of 2.6 %-units decrease in rumen cell wall digestion is only partly translated into a decreased production of VFA and CH_4 because only part of dietary DM is attributable to cell walls, and hence MEF and MCF were reduced by only 1%.

However, a difference in the fraction of unsaturated long chain fatty acids from 20% to 80% is not representative for n estimate of the impact of uncertainty around this factor on predicted rumen fermentation and CH_4 production. An uncertainty of 5% in the degree of saturation seems more realistic and for this reason the effect of fat saturation on uncertainty of MEF and MCF will remain very small, unless there are additional effects on the microbial population (e.g. inhibition of protozoal activity and methanogens) which have not yet been represented in the model.

Finally, a predicted decline in rumen cell wall digestion with 2.6 %-units indicates that effects of degree of saturation of fat may be confounded with those of the DM intake realized, meeting of VEM-requirements and milk production realized. In order to comply with the WUM-results these aspects were not taken into account or investigated in the present study. This is prerequisite however when analysing in vivo observations of effects of the amount and type of fat added to dairy rations. The size of effect of dietary fat increases with dietary fat content; furthermore it depends on diet type. Dijkstra *et al.* (2000) predicted for a dietary fat content of 8% that a shift from 20% to 80% unsaturated long chain fatty acids in fat decreased rumen cell wall digestion with 6.5% and 1.8 %-units of digestion in a forage-poor and a forage-rich diet, respectively.

In the model unsaturated fatty acids in particular are presumed to have a negative effect on cell wall degradation. The model does not distinguish between different types of unsaturated fatty acids. It is known, however, that unsaturated fatty acids differ in their effect on digestion and metabolism ((Giger-Reverdin *et al.*, 2003). For example, a small amount of DHA (C22:n-3) already caused a strong shift in metabolism and milk fat content (Boeckaert *et al.*, 2008). Also some saturated fatty acids, such as C12:0 and C14:0, may have a detrimental effect on rumen fermentative metabolism (Dohme *et al.* 2004), which is not represented in the model used in the present study. Finally, a high dietary fat content is often accompanied by a stronger decrease in feed intake than might be expected on the basis of the higher VEM value of this fat. Also such an effect is not taken into account in the model. In current practice, dairy farmers are still reluctant however to apply diets with a high fat content (more than 60 g of fat/kg DM is rare). Therefore, the fact that such effects are not represented in full detail in the model will not have a strong effect on calculations for common rations which are not high in fat. Moreover, it will have minor effects on estimates of the uncertainty of MEF and MCF predictions.

Changing rumen acidity (*acidity & fat content* and *acidity & fat saturation*) from pH 6.5 to pH 6.0 or 5.5 (given as a model input instead of prediction of pH according to the current ER protocol) resulted in a 6 and 28 decrease of %-units of rumen digestion of cell walls. This means that a large part of the decrease of MEF and MCF was caused by a decreased of cell wall fermentation. However, cell walls take about 50% of dietary DM. A large part of the effect of increased acidity on MEF and MCF is caused by a changed profile of VFA-production which generates less CH_4 (see Section 3.5.1 and 3.5.2). A shift of 1.0 pH unit was tested here but an uncertainty of the size of 0.1 pH unit is more realistic, which infers a 3% uncertainty of MEF and MCF prediction. This corresponds to the results discussed in Section 3.5.1.

An increase of the dietary fat content (*acidity & fat content*) in combination with a pH 6.5, 6.0 or 5.5 on average a decrease of MEF and MCF of 9% and 7%, respectively. The decrease was strongest with the combination of pH 5.5 and 80% unsaturated long chain fatty acids in fat (decrease of 11% and 9% of MEF and MCF, respectively). The change in size of the effects of an increased dietary fat content was opposite with 20% or 80% unsaturated long chain fatty acids in dietary fat. A decline of pH from 6.5 to 5.5 resulted in a diminishing decrease of MEF from 8,9% to 7.2% with 20% unsaturated fatty acids, whereas with 80% unsaturated fatty acids this decrease was growing from 9.1% to 11.0%. Effects on MEF and MCF can also be expressed as an effect of a changed proportion of unsaturated long chain fatty acids in fat (*acidity & fat saturation*). With a decrease of pH from 6.5, to 6.0, to 5.5, an increased in degree of saturation caused a decrease of MEF of 0.2%, 1.3% and 4.4%, respectively, at a high dietary fat content. For the lower fat content of the reference diet this effect was much smaller with 0.0%, 0.3% and 1.0%, respectively, These effects can be explained by the fact that at pH 5.5 the predicted cell wall degradation, already strongly inhibited, is more sensitive for increased dietary fat content. This sensitivity increases further with an increase of the amount of unsaturated long chain fatty acids in the rumen. This occurs under circumstances with an elevated dietary fat content as well as an increased fraction of unsaturated long chain fatty acids in fat.

The uncertainties of the influencing factors rumen acidity, dietary fat content and degree of saturation of dietary fat for the reference situation is far smaller than tested here. Uncertainties are rather of a size of a 2.5 g fat / kg dietary DM (about six times smaller than the effect tested), of 5% of the percentage if unsaturated fatty acids in dietary fat (about six times smaller than effect tested) and 0,1 pH unit (0 times smaller than the effect tested). When the calculated uncertainties of MEF and MCF are decreased in proportion, the size of uncertainty remaining is small (Figure 8). It is concluded that the effect of rumen fat metabolism is insignificant compared to the effect of rumen acidity on cell wall degradation and on the amounts and types of VFA produced. The latter results determine most of the estimated uncertainty of MEF and MCF predictions.

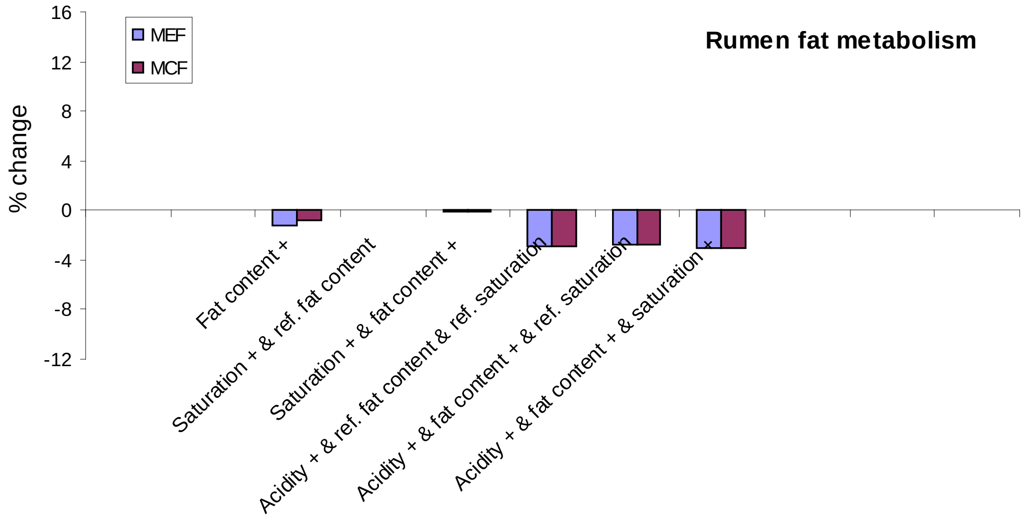

Figure 8. Overview of the effects of an increase of 25 g fat/kg DM in the fat content of concentrates, of an exchange with sugars and starch in proportion to their contributions to concentrates DM, of a 10% increased degree of saturation of long chain fatty acids in fat, and of an increased acidity of rumen contents, on predicted MEF and MCF values. Effects are expressed as changes (in %) in predicted MEF and MCF values compared to those calculated for the reference situation.

4 Conclusions

The total uncertainty in the values of MEF and MCF predicted by the model is determined by uncertainties concerning model inputs as well as model representation. Table 5 summarizes the uncertainties investigated and their effects on MEF and MCF. Of all the uncertainties for model inputs that we tested, those in feed intake (relating to the uncertainty in milk production level per cow, the VEM value of dietary components, and feeding losses) caused a 5% uncertainty in the predicted MEF (Results Section 3.4.1). The uncertainty in the partition of VEM intake over various dietary components (Results Section 3.4.2) and the uncertainty in the chemical composition of dietary components (Results Section 3.4.3) caused additional uncertainties in the MEF of 1% and 2%, respectively. This brings the total uncertainty in the predicted MEF to around 8%, and that in the MCF to around 4.5% (Table 5).

Uncertainties associated with the model representation can be subdivided into the uncertainty in some crucial model parameters and the uncertainty in crucial internal calculation rules. Uncertainty in the model parameters (Results Section 3.5.1) caused an uncertainty of 3% in the MEF. An additional uncertainty of 5% was estimated in relation to the uncertainty in VFA stoichiometry. The content and characteristics of dietary fat and fermentation in the large intestine had a minor impact on the uncertainty in MEF and MCF predictions, compared to the impact of model inputs, model parameters and VFA stoichiometry. Assuming a 1% uncertainty in the MEF associated with dietary fat content and rumen fat metabolism, the total uncertainty in the MEF associated with model representation then becomes 9%. The corresponding total uncertainty in the MCF amounts to about 11%. A smaller fraction of the uncertainty was attributed to MEF, because this is highly dependent on the amount of DM fermented and hence on the amount of fermentable DM consumed by the cow. Since this effect overlaps to a large extent with the estimated uncertainty associated with feed intake, we decided to allocate only part of the total uncertainty calculated for model representation. The MCF appears to be three times less sensitive than MEF to the effects of feed intake, so there is also less overlap with uncertainties related to model representation. For this reason, the uncertainty associated with model representation was fully allocated to MCF (see Table 5 & Table 6).

The two types of uncertainties (those associated with model inputs and those associated with model representation) result in a total uncertainty of 19% for predicted MEF, and of 15.5% for predicted MCF (see Table 5 & Table 6).

Table 5. Overview of factors contributing to the uncertainty in estimated enteric CH_4 production by dairy cows

Factor investigated	**MEF (kg CH_4/cow/yr)**	**MCF (CH_4 as % of gross energy intake)**
Model inputs		
Feed intake	5%	1.5%
Feed composition	1%	1%
Chemical composition	2%	2%
Model representation		
Model parameters	5%	5%
VFA stoichiometry	5%	5%
Large intestine fermentation	0%	0%
Dietary fat	1%	1%
Total	**19%**	**15.5%**

Table 6. Summary of uncertainties as a result of error in model inputs, error in some internal model parameters or error in some internal calculation rules, expressed in absolute values of the methane emission factor (kg methane/cow/year; MEF) and in methane as a percentage of gross energy (GE) intake (MCF). This table shows the average magnitude of changes in MEF and MCF without a sign that indicates the direction of change. The reader is referred to Table 4 and Figures 3 to 8 for an indication of the sign of change in MEF and MCF obtained with a specific sign of uncertainty of model inputs, of some internal model parameters or of some internal calculation rules. For further explanation see Table 4.

		MEF ($kgCH_4$/cow/yr)	**MCF** (% GE intake)
Predicted value for reference		129.4	5.91
Term used for change with respect to reference	**Size of uncertainty or choice of alternative representation**	**Error or change (%) of predicted MEF**	**MCF**
Feed intake (unequal DM and VEM intake)			
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%
	Realistic estimate of contribution to uncertainty	*5%*	*1.5%*
Partition dietary components (equal VEM intake)			
VEM intake grass herbage (exchange grass & maize silage, conc.)	10% total DM intake	0.9%	1.0%
VEM intake grass herbage/grass silage (exchange grass silage)	10% total DM intake	0.4%	0.9%
VEM intake maize silage (exchange grass herbage, grass silage)	10% total DM intake	0.7%	0.7%
VEM intake conc. (exchange grass herbage, grass & maize silage)	10% total DM intake	0.4%	0.2%
	Realistic estimate of contribution to uncertainty	*1%*	*1%*

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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)	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)	10 g CP/kg DM grass silage	1.0%	0.7%
Starch maize silage (exchange cell walls)	25 g starch/kg DM maize silage	0.0%	0.4%
Starch/sugar concentrates (exchange sugars)	50 g starch/kg DM concentrates	0.5%	0.5%
Starch/cell walls concentrates (exchange cell walls)	50 g starch/kg DM concentrates	0.1%	0.0%
Starch/sugar&CP concentrates (exchange sugar, CP)	10 g CP/kg DM concentrates	0.1%	0.2%
	Realistic estimate of contribution to uncertainty	*2%*	*2%*
Model parameters			
Particle passage	0.1 /day	1.1%	1.1%
Fluid passage	0.2 /day	0.6%	0.6%
Rumen volume	10 litre	1.0%	1.0%
Acidity	0.1 pH unit	3.1%	3.1%
	Realistic estimate of contribution to uncertainty	*5%*	*5%*
Internal calculation rules			
VVVZ-stoichiometry Bannink 2000	alternative stoichiometry forage-rich diets	10%	10%
VVZ-stoichiometry Murphy 1982	alternative stoichiometry forage-rich diets	18%	18%
	Realistic estimate of contribution to uncertainty	*5%*	*5%*
No large intestine	neglecting large intestine	8%	8%
Passage large intestine	50% change	0%	0%
Fat content	fat content concentrates 15 g/kg DM	1.2%	0.8
Fat saturation	degree of saturation fatty acids 5%	0.1%	0.1%
Acidity & fat	0.1 pH unit (in combination with fat)	3.1%	3.1%
	Realistic estimate of contribution to uncertainty	*1%*	*1%*
	Summation of all uncertainties	**19%**	**16%**
	Eventual estimate [1] of uncertainty	**15%**	**13%**

[1] Uncertainties are not 100% additive (see text Chapter 4 for further explanation).

Let us assume that the uncertainties investigated do not influence each other (i.e. are not related by some underlying mechanism) and that they are fully independent. Let us also assume that the variation in these uncertainties is fully random and follows a normal distribution. In that case, the total uncertainty could be calculated as the square root of the sum of individual squared uncertainties. This would lead to far lower uncertainties, viz. only 9% and 8% for MEF and MCF, respectively. However, the uncertainties tested are not independent, because of the nature of the model and because they are 'connected' through the mechanism represented by the model. This means that the individual uncertainties we tested are expressed mainly, but not fully, in an additive manner in the total uncertainty in MEF and MCF. Because the assumption that they are additive does not fully hold, the total summed uncertainties of 19% and 15.5% for MEF an MCF, respectively, have to be adjusted downwards (see the conclusions in Sections 3.4.1, 3.4.2, 3.4.3, 3.5.1, 3.5.2 and 3.5.3). Therefore, intermediate uncertainty values of 15% and 13% for MEF and MCF, respectively, seem more realistic, and these are currently used in the Dutch ER protocol.

The results of our simulation studies demonstrate that the accuracy of MEF prediction is mainly determined by the accuracy of the assumptions about feed intake (the VEM value of dietary DM and in particular that of grass products, and whether VEM intake meets the assumed VEM requirement) and the stoichiometry of VFA production. Both cause an uncertainty of 5%. The internal model parameters (rumen acidity in particular) as well as the chemical composition of dietary components (sugar and protein content of grass silage in particular) contribute less to the uncertainty, viz. 3% and 2%, respectively. All other factors we investigated contributed less than 1% to the uncertainty in the MEF.

The greatest part of the uncertainty in the MCF also appeared to depend on the uncertainty associated with the stoichiometry of VFA production, together with the uncertainty in the model parameters (rumen acidity in particular). Each contributed 5% to the overall uncertainty, while the chemical composition of dietary components contributed 2%, and all other factors investigated contributed 1.5% or less.

The uncertainty in the stoichiometry of VFA production therefore seems to be the most important factor determining the accuracy of the prediction of both MEF and MCF in the reference situation currently under study, followed by the acidity of the digesta in the rumen. The uncertainty in the feed intake is only important with respect to the accuracy of MEF prediction. The uncertainty associated with the partition of individual dietary components, dietary fat content and fat characteristics, and the contribution of the large intestine appeared the least important in terms of the accuracy of current MEF and MCF predictions in the Tier 3 approach for CH_4 emission by dairy cows used in the Dutch ER protocol.

A final point of discussion is the extent to which the assumptions made in the current methodology of the national inventory on nutrition and performance of dairy cows (www.cbs.nl) are correct under various production conditions. This inventory uses the assumptions of the system of net energy of lactation (VEM system; Van Es, 1978; Tamminga *et al.*, 2004). However, these underlying assumptions may not always hold, and the feed energy values analysed by commercial laboratories may also be inappropriate for the production conditions to be studied. Both aspects deserve further evaluation.

An indication of the limitations of the current methodology used to derive the diet for the average dairy cow in the Netherlands, based on the VEM system, are the results obtained by Dijkstra *et al.* (2008b). Their study analysed grass herbage rations with an adapted version of the Tier 3 model, and compared the model predictions with the expectations according to the VEM system. The results demonstrated that a nutrient-based evaluation based on simulated fermentative and digestive processes produced more accurate prediction of the milk yield. This suggests that for these production conditions (mainly grass herbage), the VEM system might be less accurate than an approach similar to the current Tier 3 model. Another example is an analysis of the effect of grass quality and type of grass product (grass herbage or grass silage) on CH_4 emission in dairy cattle (Bannink *et al.*, 2010). This study also clearly revealed the profound effect of assumptions about grass characteristics and feeding value on simulated enteric CH_4 production. This indicates that VEM values may also be different from those analysed by commercial laboratories for practical purposes. A final example is the fact that large changes in dietary composition may result in the reported feeding values not being additive, whereas the current methodology (VEM system) assumes they are. For example, excessive starch intake, or a diet producing an acidic rumen environment, may be detrimental to diet digestibility and hence milk production. Such specific effects are not taken into account by the VEM system, which treats VEM values in all dietary components independently and regards them as fully additive, irrespective of the level of DM intake or the composition of dietary DM. The VEM system does, however, apply a general correction factor for the VEM requirement to accommodate for the effect of level of feed intake.

To address such problems, a model similar to the current Tier 3 model (Dijkstra *et al.*, 2008b) may be used to predict not only enteric CH_4 emission (the present study), but also feed digestion in the rumen, small intestine and large intestine, as well as the supply of individual nutrients absorbed and the consequences for milk synthesis and apparent feed utilization. Such an extended use of the current Tier 3 model (or an updated version of it) would serve two purposes. First, it would indicate whether the predictions about digestion, dietary energy value and milk synthesis obtained with the current Tier 3 model match the assumptions about nutrition and performance of dairy cows of the VEM system used in the current national inventory. Second, it would provide a more accurate instrument to simulate effects on enteric CH_4 emission under highly specific production conditions, as opposed to the aim of predicting for the national average condition. For the national average production conditions for dairy cows, the current methodology probably suits the purpose just as well as such an extended use of the Tier 3 model would. The current use is also to be preferred from a pragmatic viewpoint, because it requires data which are readily available in practice. However, if the focus is on much more specific production conditions, e.g. regional differences or extreme dietary options, a more extended use of the Tier 3 model is preferable, which is able to evaluate effects on digestive and fermentative processes, on nutrient supplies to cows and on milk synthesis. The current Tier 3 model is probably the more appropriate candidate model to address such specific questions.

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Appendix 1 Methane emission in dairy cows from 1990 till 2008 according to the Dutch Tier 3 approach (published in the Dutch National Inventory Report 2010, Van der Maas *et al*, 2010)

Introduction

In 2009 historic data of feed intake, milk production and composition of the average Dutch dairy diet from the year 1990 till the year 2007 were revised. For this reason, previous calculations of enteric CH_4 emission in dairy cows were revised as well and presented in the NIR 2009. In 2010 the time series was extended with the year 2008 and published in the NIR 2010.

The method of obtaining estimates of enteric CH_4 emission is described in Chapter 2 of this report. This Appendix describes the input data and results obtained with the revised methodology (NIR 2009: Van der Maas *et al.,* 2009; NIR 2010: Van der Maas *et al*, 2010).

Appendix 2 describes the input data and results used with the previously used methodology (NIR 2008: Van der Maas *et al.*, 2008).

The revision of methodology included the following changes:

1. Feed losses of roughages, concentrates and by-products were taken into account according the methodology adopted by the Working group Unifying Manure and excretion data (WUM; www.cbs.nl). These losses were not taken into account with the previous NIR publications (Smink *et al.*, 2005; Appendix 2).
2. There was a discrepancy in methodology to estimate the requirement for net energy of lactation with studies on N excretion by lactating cows (Tamminga *et al.*, 2004) and methodology applied for by the Working group Unifying Manure and excretion data (WUM; www.cbs.nl). The former took into account some additional factors which are relevant under practical conditions and which increase estimated feed requirements. This increment of feed requirement matches insights obtained from observations on farms in practice (Tamminga *et al.*, 2004) and from energy balance trials which indicate a 6% higher energy requirement for maintenance than formulated according to energy evaluation systems (Dijkstra *et al.*, 2008b).
   In order to have a uniform methodology applied for the whole time series, estimates of CH_4 emission were re-calculated for the whole time period of 1990 till 2008 published in the NIR 2010 according to the updated methodology on calculation of energy requirement and feed intake.
3. A correction was included for the ammonia-N fraction of N in the crude protein fraction This correction was not implemented with previous NIR publications (Smink *et al.*, 2005; Appendix 2). The impact on estimated CH_4 emission remains relatively small compared to that associated with the revisions indicated in 1. and 2.

Feed intake data

Data of feed intake were derived from the recently adapted database by WUM (Van Bruggen, 2010; www.cbs.nl). The occurrence of feed losses and the fraction of ammonia-N in crude protein in silages were taken into account.

	Feed intake (kg dry matter / cow / year)							Feed intake kg dry matter cow / day	Gross energy intake (MJ / cow / day)
	Grass herbage	Grass silage	Maize Silage	Concentrates standard	Concentrates Protein-rich	By-products [1]	Total		
1990	1747	1257	852	1261	270	144	5532	15.16	280.2
1991	1893	1351	699	1251	274	103	5570	15.26	280.5
1992	2169	1023	729	1356	239	60	5574	15.27	281.1
1993	1874	1291	884	1287	259	105	5702	15.62	288.2
1994	1683	1283	1119	1341	291	105	5823	15.95	294.9
1995	1749	1057	973	1449	366	185	5779	15.83	292.9
1996	1729	1133	941	1461	385	115	5765	15.79	292.0
1997	1756	1001	1074	1437	344	264	5875	16.10	297.2
1998	1051	1433	1561	1273	420	216	5953	16.31	301.9
1999	1601	1381	1226	1212	367	188	5976	16.37	302.6
2000	1261	1549	1382	1294	288	295	6069	16.63	306.5
2001	1517	1520	1298	1357	248	200	6141	16.82	310.8
2002	1454	1351	1492	1380	200	208	6084	16.67	308.3
2003	849	2115	1486	1397	229	233	6310	17.29	318.7
2004	1044	1931	1512	1446	221	202	6356	17.42	320.9
2005	1270	1773	1516	1390	193	212	6354	17.41	319.9
2006	1358	1714	1619	1386	184	212	6474	17.74	326.8
2007	1328	1923	1556	1412	186	186	6591	18.06	333.0
2008	1248	1860	1560	1312	402	189	6571	18.00	332.4

[1] For wet by-products, the assumption was made that wet brewer's grains, by-products from the potato processing industry and pressed beet pulp contributed 25%, 25% and 50%, respectively, to the total dry matter intake of by-products from 1990 to 2003; contributions of 25%, 40% and 35%, respectively, were assumed from 2004 to 2006, and contributions of 31%, 42% and 27%, respectively, for 2007 and of 32%, 43% and 25% for 2008. Estimates were obtained from data generated by WUM (www.cbs.nl).

Chemical composition of dietary components

Chemical composition (g/kg dry matter) and feeding value (VEM/kg dry matter) are listed in the tables below, successively for grass herbage, grass silage, maize silage, standard concentrates and protein-rich concentrates (values in italics were assumed rather than analysed). Data retrieved from Smink *et al*. (2005) or collected by the Working group Unifying Manure and excretion data (WUM; www.cbs.nl).

Grass herbage

	VEM	Ash	Crude protein	Crude fat	NDF	Sugars	Fermentation Products
1990		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		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*[1]	117	*0*
2005	975	107	207	*40*	*475*[1]	120	*0*
2006	957	104	200	*40*	*475*[1]	109	*0*
2007	930	104	191	*40*	*475*[1]	113	*0*
2008	932	105	202	*40*	511[2]	102	*0*

[1] Value based on NDF analysis in 2003.

[2] Only since 2008 has an estimate for NDF been available from CBS data.

Grass silage

	VEM	Ash	Crude protein	Fraction ammonia in CP (%CP)	Crude fat	NDF	Sugars	Fermentation products
1990	868	119	189	*6*	*40*	*493*	*78*	*50*
1991	838	125	177	*6*	*40*	*493*	*78*	*50*
1992	857	121	184	*6*	*40*	*493*	*78*	*50*
1993	861	*118*	*179*	*6*	*40*	*493*	*78*	*50*
1994	863	*118*	*179*	*6*	*40*	*493*	*78*	*50*
1995	839	115	179	*6*	*40*	*493*	90	*50*
1996	874	134	209	*6*	*40*	*493*	58	*50*
1997	845	125	183	*6*	*40*	*493*	64	*50*
1998	868	123	176	*6*	*40*	479	63	*50*
1999	879	111	179	*6*	*40*	463	101	*50*
2000	877	120	178	*6*	*40*	493	65	*50*
2001	893	106	174	*6*	*40*	486	108	*50*
2002	863	116	167	*6*	*40*	510	74	*50*
2003	847	112	159	*6*	*40*	530	82	*50*
2004	896	111	173	9.4	*40*	489	78	*50*
2005	897	109	160	8.8	*40*	481	99	*50*
2006	891	101	168	8.0	33	504	98	*50*
2007	876	106	161	8.4	37	511	83	*50*
2008	888	107	161	8.0	40	497	88	*50*

	VEM	Ash	Crude protein	Fraction ammonia in CP (%CP)	Crude fat	NDF	Sugars	Starch	Fermentation products
Maize silage									
1990 – 2003		*42*	*74*	*6*	*30*	*433*	*15*	*371*	*35*
2004	960	41	71	7.2	30	412	13	348	*35*
2005	940	41	71	8.1	30	432	13	332	*35*
2006	977	40	79	10.2	31	382	14	356	*35*
2007	963	38	70	7.7	37	393	13	342	*35*
2008	962	39	73	5.3	36	388	13	342	*35*
Standard concentrates									
1990 – 2003		*100*	*180*	*0*	*50*	*320*	*100*	*250*	*0*
2004	940	*100*	178	*0*	*50*	*320*	*100*	*250*	*0*
2005	940	*100*	179	*0*	*50*	*320*	*100*	*250*	*0*
2006	940	*100*	179	*0*	*50*	*320*	*100*	*250*	*0*
2007	*940*	*100*	174	*0*	*50*	*320*	*100*	*250*	*0*
2008	*940*	*100*	166	*0*	*50*	*320*	*100*	*250*	*0*
Protein-rich concentrates									
1990 - 2003		*100*	*330*	*0*	*50*	*270*	*70*	*180*	*0*
2004	940	*100*	244	*0*	*50*	*270*	*70*	*180*	*0*
2005	940	*100*	244	*0*	*50*	*270*	*70*	*180*	*0*
2006	940	*100*	241	*0*	*50*	*270*	*70*	*180*	*0*
2007	*940*	*100*	239	*0*	*50*	*270*	*70*	*180*	*0*
2008	*940*	*100*	245	*0*	*50*	*270*	*70*	*180*	*0*

Degradation characteristics of feeds

The model requires input data for in situ degradation characteristics of protein, starch and NDF (cell wall material). A distinction is made between the washable fraction, W, the non-washable, but degradable fraction, D, and the non-washable, undegradable fraction, U. The D fraction requires an estimate of the fractional degradation rate, kd. The WUM data offer no information on such characteristics, and some realistic values were derived from data from previous experiments conducted by ASG. The values listed below were assumed to be realistic estimates.

Grass herbage			
Crude protein:	W=15.0%	D=77.5%	kd=9.0%/h
Cell walls (NDF):	W=0.0%	D=87.5%	kd=6.0%/h
Grass silage			
Crude protein:	W=35.0%	D=55.0%	kd=5.0%/h
Cell walls (NDF):	W=0.0%	D=82.5%	kd=4.0%/h.
Maize silage			
Crude protein:	W=57.5%	D=22.5%	kd=2.0%/h
Cell walls (NDF):	W=0.0%	D=60.0%	kd=2.0%/h
Starch:	W=30.0%	D=70.0%	kd=10.0%/h
Standard concentrates			
Crude protein:	W=32.5%	D=62.5%	kd=6.5%/h
Cell walls (NDF):	W=0.0%	D=85.0%	kd=7.5%/h
Starch:	W=57.5%	D=42.5%	kd=10.0%/h
Protein-rich concentrates			
Crude protein:	W=22.5%	D=75.0%	kd=6.0%/h
Cell walls (NDF):	W=0.0%	D=80.0%	kd=6.0%/h
Starch;	W=30.0%	D=70.0%	kd=8.0%/h

Results

The table below indicates the calculated enteric CH_4 emission by dairy cows in the Netherlands for the whole time series of 1990 to 2008.

Feed intake (DM in kg dry matter/cow/year, and GE in MJ/cow/year), methane production and milk production (as reported by WUM) (in kg fat-corrected milk/cow/day; FCM=fat-corrected milk)

	Feed intake		Milk production	Methane		
	kg DM/cow/yr	**MJ/cow/d**	**kg FCM/cow/d**	**kg/cow/yr**	**% of GE intake**	**g/kg FCM [1]**
1990	5532	280.17	17.17	110.5	6.03	17.62
1991	5570	280.53	17.36	111.2	6.04	17.55
1992	5574	281.07	17.59	111.9	6.07	17.44
1993	5702	288.17	18.02	113.9	6.03	17.32
1994	5823	294.87	18.51	115.6	5.98	17.11
1995	5779	292.92	18.91	115.8	6.02	16.77
1996	5765	292.02	19.17	113.5	5.92	16.21
1997	5875	297.18	19.47	117.0	6.00	16.46
1998	5953	301.94	19.68	116.9	5.90	16.28
1999	5976	302.62	19.91	119.1	6.00	16.39
2000	6069	306.52	20.76	120.0	5.97	15.84
2001	6141	310.82	21.05	122.1	5.99	15.89
2002	6084	308.25	20.77	120.2	5.95	15.86
2003	6310	318.68	21.26	123.3	5.90	15.90
2004	6356	320.94	21.50	124.8	5.93	15.90
2005	6354	319.87	21.89	126.3	6.02	15.81
2006	6474	326.79	22.38	127.8	5.96	15.64
2007	6591	332.99	22.82	129.4	5.92	15.53
2008	6571	332.44	22.92	128.3	5.88	15.34

[1] Milk production was expressed as fat-corrected milk (FCM), similar to the results obtained before revision of the methodology presented in Appendix 2. In contrast, results in Chapter 2 are presented as fat- and protein-corrected milk because this is the more familiar unit used in the literature on dairy nutrition.

Conclusions

From the year 1990 to 2008, the yearly feed intake by Dutch dairy cows increased by 0.16 kg dry matter/cow/day, which resulted in a total increase of 18% during this whole period. The yearly increase in the amount of fat-corrected milk produced per cow was 0.33 kg/cow/year, which led to a total increase of 32% during the whole period. The calculated yearly emission of methane increased by 1.1 kg methane/cow/year, corresponding to a total increase of 16% during the whole period. This means that feed intake and milk production increased at a faster rate than methane emission.

As a result, the yearly decrease in methane emission per kg of fat-corrected milk was 0.13 kg methane/kg fat-corrected milk/year, corresponding to a total decrease of 12% during the whole period of 1990 to 2008.

Appendix 2 Calculations of methane emission in dairy cattle according to the Tier 3 approach from 1990 till 2006 according to the previous methodology

Data based on reports delivered in NIR 2006, NIR 2007 and NIR 2008: Van der Maas et al., 2008 by A. Bannink (Animal Sciences Group, Wageningen UR, Lelystad) to PBL.

Introduction

Comparable to the methods described by Smink *et al.* (2005) the enteric CH_4 emission in dairy cows was calculated for the rime period of 1990 till 2006. This Appendix describes the model input used and the results obtained. These results have been included in previous NIR publications (NIR 2008: Van der Maas *et al.*, 2008).

In a more recent NIR publications (NIR 2009: Van der Maas *et al.,* 2009; NIR 2010: Van der Maas *et al*, 2010) these results have been recalculated because os a revises methodology (see Appendix 1).

Feed intake data

Data of feed intake (in dry matter, DM) were derived from WUM (www.cbs.nl) and listed by Smink *et al.* (2005) until the year 2004. The data were obtained with the previous methodology (also see Appendix 1), not corrected for feed losses.

	Feed intake (kg DM/cow/year)							**Feed intake (kg DM cow/day)**
	Grass herbage	**Grass silage**	**Maize Silage**	**Concentrates standard**	**Concentrates Protein-rich**	**By-products** [1]	**Total**	
1990	1484	1252	902	1285	276	166	5365	14.70
1991	1637	1343	741	1275	279	123	5399	14.79
1992	1843	959	818	1411	244	95	5370	14.71
1993	1671	1277	935	1313	264	79	5539	15.18
1994	1396	1280	1182	1368	297	124	5646	15.47
1995	1480	1039	1026	1476	373	211	5606	15.36
1996	1462	1137	994	1489	392	135	5609	15.37
1997	1485	972	1136	1458	351	298	5701	15.62
1998	999	1523	1348	1359	379	241	5849	16.02
1999	1266	1498	1283	1248	373	212	5881	16.11
2000	994	1670	1443	1351	292	238	5988	16.41
2001	1244	1639	1354	1393	252	222	6104	16.72
2002	1045	1706	1420	1422	208	229	6030	16.52
2003	732	2171	1594	1422	234	258	6411	17.56
2004	808	2209	1569	1456	223	223	6487	17.77
2005	770	2362	1643	1326	185	229	6515	17.85
2006	653	2615	1727	1273	178	218	6664	18.26

[1] As regards wet by-products, it was assumed that wet brewer's grains, by-products from the potato-processing industry and pressed beet pulp contributed 25%, 25% and 50%, respectively, to the total dry matter intake of by-products between 1990 and 2003; the contributions for 2004 to 2006 were assumed to be 25%, 40% and 35%, respectively. Estimates were obtained from data generated by WUM (www.cbs.nl).

Chemical composition of dietary components

The chemical composition (g/kg dry matter) and the feeding value (VEM/kg dry matter) are listed in the tables below for, successively, grass herbage, grass silage and maize silage, and concentrates of grass silage (source: BLGG; WUM, Den Boer and Bakker, 2005). Values are in units or gram/kg dry matter. Average nutrient contents were used for missing values in the table. Data up to 2004 were obtained from Smink *et al*. (2005).

Grass herbage[1]

	VEM	Ash	Crude protein		Crude fat	NDF	Sugar	FP
1989		99	246		*40*	*479*	*97*	*0*
1990		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		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*[2]	117	*0*
2005	975	107	207		*40*	*475*[2]	120	*0*
2006	957	104	200		*40*	*475*[2]	109	*0*

[1] Numbers in italics were estimated or assumed to equal the average of measured values in the period of 1990-2003.

[2] Value based on NDF-analysis in 2003; only since 2008 has an estimate for NDF content been available from CBS data.

Grass silage[1]

	VEM	Ash	Crude protein	Fraction ammonia (% RE)	Crude fat	NDF	Sugar	FP
1989	911	109	182	*6*	*40*	*493*	*78*	*50*
1990	868	119	189	*6*	*40*	*493*	*78*	*50*
1991	838	125	177	*6*	*40*	*493*	*78*	*50*
1992	857	121	184	*6*	*40*	*493*	*78*	*50*
1993	861	*118*	*179*	*6*	*40*	*493*	*78*	*50*
1994	863	*118*	*179*	*6*	*40*	*493*	*78*	*50*
1995	839	115	179	*6*	*40*	*493*	90	*50*
1996	874	134	209	*6*	*40*	*493*	58	*50*
1997	845	125	183	*6*	*40*	*493*	64	*50*
1998	868	123	176	*6*	*40*	479	63	*50*
1999	879	111	179	*6*	*40*	463	101	*50*
2000	877	120	178	*6*	*40*	493	65	*50*
2001	893	106	174	*6*	*40*	486	108	*50*
2002	863	116	167	*6*	*40*	510	74	*50*
2003	847	112	159	*6*	*40*	530	82	*50*
2004	896	111	173	9.4	*40*	489	78	*50*
2005	897	109	160	8.8	*40*	481	99	*50*
2006	891	101	168	8.0	33	504	98	*50*

[1] Numbers in italics were estimated or assumed to equal the average of measured values.

[1]	VEM	Ash	Crude protein	Fraction NH_3 (%RE)	Crude fat	NDF	Sugar	Starch	FP
Maize silage									
1990 - 2003		*42*	*74*	*6*	*30*	*433*	*15*	*371*	*35*
2004	960	41	71	7.2	30	412	13	348	*35*
2005	940	41	71	8.1	30	432	13	332	*35*
2006	977	40	79	10.2	31	382	14	356	*35*
Standard concentrate									
1990 - 2003		*100*	*180*	*0*	*50*	*320*	*100*	*250*	*0*
2004	940	*100*	178	*0*	*50*	*320*	*100*	*250*	*0*
2005	940	*100*	179	*0*	*50*	*320*	*100*	*250*	*0*
2006	940	*100*	179	*0*	*50*	*320*	*100*	*250*	*0*
Protein rich concentrate									
1990 - 2003		*100*	*330*	*0*	*50*	*270*	*70*	*180*	*0*
2004	940	*100*	244	*0*	*50*	*270*	*70*	*180*	*0*
2005	940	*100*	244	*0*	*50*	*270*	*70*	*180*	*0*
2006	940	*100*	241	*0*	*50*	*270*	*70*	*180*	*0*

[1] Numbers in italics were estimated or assumed to equal the average of measured values in the 1990-2003 period.

Results

The table shows the result of calculations of CH_4 emission by dairy cows in the Netherlands from 1990 to 2006. Feed intake (DM in kg dry matter/cow/yr, and GE in MJ/cow/yr) and enteric CH_4 emission (MEF in kg CH_4/cow/yr; MCF in CH_4 energy as % of GE intake).

Year	Feed intake				Methane	
	kg DM/cow/yr	MJ/cow/yr	kg/cow/yr MEF	MJ/cow/yr	% GE MCF	g CH_4/kg FCM [1]
1990	5365	98733	107.7	5994	6.07	16.83
1991	5399	98827	108.1	6016	6.09	16.68
1992	5370	98554	108.4	6032	6.12	16.64
1993	5539	101784	110.8	6166	6.06	16.52
1994	5646	103941	112.4	6255	6.02	16.49
1995	5606	103350	112.7	6272	6.07	16.16
1996	5609	103273	110.7	6160	5.96	15.70
1997	5701	104938	114.0	6344	6.04	15.78
1998	5849	107478	115.4	6422	5.97	15.95
1999	5881	108197	117.1	6517	6.02	15.84
2000	5988	109876	117.9	6561	5.97	15.04
2001	6104	112179	121.1	6739	6.01	15.48
2002	6030	110624	118.8	6611	5.98	15.53
2003	6411	117497	124.6	6934	5.90	15.01
2004	6487	118512	125.9	7001	5.91	15.98
2005	6515	118616	127.6	7093	5.98	15.93
2006	6664	121824	129.4	7202	5.91	15.79

[1] Milk production was expressed as fat-corrected milk (FCM), similar to the results obtained after revision of the methodology presented in Appendix 1. In contrast, results in Chapter 2 are presented as fat- and protein-corrected milk because this is the more familiar unit used in the literature on dairy nutrition.

Verschenen documenten in de reeks Werkdocumenten van de Wettelijke Onderzoekstaken Natuur & Milieu vanaf 2009

Werkdocumenten zijn verkrijgbaar bij het secretariaat van Unit Wettelijke Onderzoekstaken Natuur & Milieu, te Wageningen. T 0317 – 48 54 71; F 0317 – 41 90 00; E info.wnm@wur.nl
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