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Interactions between enteric methane and nitrogen excretion in dairy cows

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Next to dry matter (DM) intake, nutritional factors cause considerable variation in methane (CH_4) emitted and nitrogen (N) excreted per kg of DM intake or per kg of milk. Rumen function in particular determines CH_4 emission and concomitant (amount and site) of N excretion, including the trade-offs between them with changes in nutrition and cow characteristics. Quantification of the interaction between CH_4 and N emission hence requires quantification of effects on rumen function in particular. The models available to quantify CH_4 emission require the same types of input. The detail of questions posed determines the choice of model and the required level of detail of model inputs needed to investigate mitigation measures and the interaction between CH_4 and N emission for a specific farming case. Simulation results with a mechanistic model of enteric fermentation confirmed a profound impact of nutritional measures on both CH_4 and N emission, but also demonstrated that nutritional measures to mitigate N excretion can be associated with an increase in CH_4 emission. This result demonstrates the need to consider details on the rumen level when the aim is to quantify accurately the net effect on greenhouse gas emission for a specific case studied, which contrasts with applying generic values. As an alternative to models of quantification, on-farm measurement of emission might be pursued by sampling of excreta and air. The principle problem is that concentrations are measured which not necessarily reflect daily rates. Milk production rate is recorded on-farm however, which makes indicators based on milk composition just as promising candidates to estimate CH_4 (milk fat) or N (milk urea) emission, provided bias by variation in milk composition unrelated to CH_4 and N emission rate can be prevented.

Keywords: enteric methane, nitrogen excretion, quantifying emissions, farm indicators

Introduction

Increases in productivity by dairy production systems in the last decades were generally established with a profound increase in nitrogenous emissions to the environment. A large part of these emissions depend on nitrogen (N) excreted by ruminants. More recently, the major contribution of enteric methane (CH_4) emissions to global CH_4 emissions and its global warming potential was identified (Steinfeld *et al.*, 2006). From this viewpoint, nutritional management aiming to mitigate N and CH_4 emission per unit of milk (with or without maintaining cow productivity) is desirable. This contribution explores the relationship between N excretion and CH_4 emission in response to nutritional measures in intensive dairy systems. It is discussed why a detailed representation of rumen function is a prerequisite to explaining and making use of variation in (the type of) N excretion and in CH_4 emission. It is also considered how these emissions may be quantified by the use of predictive models, or by readily available information on-farm.

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Nutritional effects on enteric CH_4

Observed CH_4 emission in cattle ranges from 2% to 12% of gross energy intake (Johnson and Johnson, 1995), and depends on the type of cattle and animal characteristics (e.g. residual feed intake), and the type of diet or production system. Within type of cattle, the main determinant of enteric CH_4 emission is dry matter (DM) intake and the rate of enteric rumen fermentation of dietary organic matter (OM). A substantial part of observed variation in enteric CH_4 remains unexplained by DM intake however, caused by variation in the rate of methanogenesis as a function of various nutritional and animal factors. Rumen fermentation of OM, efficiency of microbial growth and type of fermented substrate converted into volatile fatty acids (VFA) are main drivers of the amount of excess hydrogen that becomes available as substrate for methanogens (Figure 1). Very low amounts of hydrogen are emitted from the animal. Although there are alternative sinks of hydrogen and some alternative microbial metabolic pathways exist that can affect hydrogen availability for methanogenesis (Ellis *et al.*, 2008; Janssen, 2010), a reasonable assumption is that (under normal

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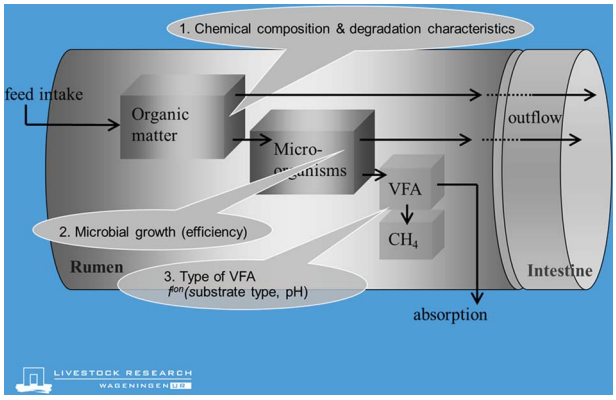


Figure 1 Rumen processes contributing to methane emission.

feeding conditions) almost all excess hydrogen, generated with the conversion of feed substrate into VFA, eventually becomes converted into CH_4. This means that the net excess of hydrogen and CH_4 production are very closely associated with the amount and profile of VFA produced (Mills *et al.*, 2001; Bannink *et al.*, 2011). Fat and long-chain fatty acids do not become fermented and do not deliver hydrogen as a substrate for CH_4 production. By hydrogenation of unsaturated fatty acids fat can actually be a relatively small sink of hydrogen and have a rather negative effect on the amount of excess hydrogen and CH_4 formed (Mills *et al.*, 2001). From the remaining fractions in fermented OM, protein delivers least CH_4 followed by starch with 10% more compared with protein, and by sugars and cell walls with around 50% more CH_4 being produced per unit of substrate converted into VFA (Bannink *et al.*, 2006 and 2008; Dijkstra *et al.*, 2011).

Nutritional measures that change the amount and profile of substrates that become fermented in the rumen must hence be expected to affect CH_4 emission. However, with manipulation of CH_4 emission by nutritional means, some potential digestive trade-offs need to be kept in mind. High-fat or high-starch diets may reduce CH_4 emission per kg of feed intake or per kg of milk produced, but they also may create less favourable rumen fermentation conditions for fibrolytic bacteria, reducing fibre digestion. A higher DM intake (e.g. by a change in diet composition with more concentrates) leads to a higher milk yield which reduces the amount of CH_4 per unit milk produced if digestibility would remain unaffected. However, with a higher DM intake the rate of OM fermentation increases and the rumen environment may become more acidic limiting fibre degradation and diet digestibility, reducing feed utilization and the marginal increase in milk yield, and as a consequence limiting the reduction in CH_4 per unit milk. Therefore, there are some potential trade-offs such as a decline of rumen degradability of feed substrates, mainly the fibre fraction of OM, because of rumen acidification and a shorter residence time in the rumen as a result of higher DM intake and faster rumen passage. These trade-offs limit rumen and total digestion and may lead to a reduced utilization of feed for milk production. Such a reduced digestibility partly counteracts the dilution effect of the amount of CH_4 originating from feed required for maintenance functions per kg milk with increase of milk yield (Mills *et al.*, 2001; Bannink *et al.*, 2011). However, a higher DM intake and rate of OM may be accompanied by a shift towards a more propionate-oriented fermentation profile that reduces the amount of CH_4 produced and compensates again the reduced diet digestibility with increase in DM intake.

In summary, variation in CH_4 produced per kg of DM intake or per kg of milk can be explained from general animal characteristics such as feed intake, feed digestion and dietary characteristics that affect OM fermentation in the rumen and the large intestine, and several trade-offs are included in the mechanisms of fermentation and digestion.

More specific nutritional effects on CH_4 may be considered, such as dietary inclusion of alternative hydrogen sinks, or perhaps even the stimulation of bacteria that compete with methanogens for hydrogen utilization if maintaining their presence in the rumen is feasible. Such nutritional measures are worthwhile to take into account if they demonstrably work *in vivo*. For example, Van Zijderveld *et al.* (2011) found a prolonged CH_4 reducing effect of nitrate as a hydrogen sink in lactating cows. With up to 20% reduction of CH_4 by addition of nitrate to the diet no increase of methemoglobin in blood plasma was observed. Hence, the inclusion of nitrate in the diet seems to be a feasible mitigation measure with an assured mode of action that promises general efficacy, without health consequences for the dairy cow when carefully managed, and without the risk of compromising milk quality.

Nutritional effects on N excretion

The excretion of N reduces when roughages or concentrates are fed with a decreased ratio of N content to energy value (net energy of lactation, NE_L). Under most production conditions, the N retained in milk protein is mainly determined by NE_L. The marginal effect of an extra amount of digested protein on milk protein yield, as established in single studies, is about three times lower than the overall effect on milk protein yield that becomes apparent across studies with different levels of NE_L intake (Cant, 2005). The retention of N therefore strongly depends on the level of feed intake, on the chemical composition of feed and on the digestibility of the chemical fractions determining NE_L. In particular, the carbohydrate fractions and protein have a variable contribution to NE_L depending on their extent and site of digestion (digestion by microbial fermentation *v.* digestion by host enzymes). Although the potential to manipulate dietary characteristics depends on farming conditions and farming strategy (e.g. milk yield per animal pursued, on-farm available roughages, costs of feed purchase), there is in principle large potential for nutritional measures to lower N : NE_L ratio to reduce N intake, to increase the fraction of ingested N that is retained and, as a consequence, to decrease the excretion and emission of N. Recently, the effect of N reducing nutritional measures was determined by Dijkstra *et al.* (2011)

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Table 1 *Description of 40 model simulations[1] including nutritional measures which reduce N excretion, and predicted milk yield and conversion of feed N to milk N averaged per nutritional measure, based on results from Reijs (2007) and Dijkstra* et al. *(2011)*

		Milk production FPCM[2](kg/day)	Feed N conversion proportion
Type of grass silage[3]			
N fertilization 350 kg N/ha	Cutting at 3000 kg DM/ha	30.6	0.25
N fertilization 350 kg N/ha	cutting at 4500 kg DM/ha	26.0	0.27
N fertilization 150 kg N/ha	cutting at 3000 kg DM/ha	28.7	0.31
N fertilization 150 kg N/ha	cutting at 4500 kg DM/ha	24.8	0.33
Grass silage replacement			
100% grass silage; 0% replacement		27.6	0.27
50% grass silage & 50% maize silage		28.3	0.32
85% grass silage & 15% straw		24.2	0.28
85% grass silage & 15% pressed beet pulp		28.7	0.29
85% grass silage & 15% potatoes		28.8	0.29
Concentrate level[4]			
60% roughages and 40% concentrates		29.5	0.28
80% roughages and 20% concentrates		25.6	0.29
Mean for all nutritional measures		27.5	0.29

[1]Dry matter intake of the complete rations was estimated using the Dutch 'Dairy CowModel' (Blok *et al.*, 2007; Zom *et al.*, 2012).
[2]FPCM, fat and protein corrected milk (4.00% fat, 3.32% protein and 4.60% lactose).
[3]Indicated are a high *v.* a low rate of N fertilization with artificial fertilizer (350 *v.* 150 kg N/ha) for an early *v.* a late cut of grass (3000 *v.* 4500 kg dry matter/ha) for a first spring cut of the grass sward for silage making. Besides artificial N fertilizer an initial application of an additional 70 kg N/ha with animal manure was assumed.
[4]Concentrate composition was assumed constant.

using a mechanistic modelling approach. The study covered a range of N reducing nutritional measures in feeding mid-lactation dairy cow. Nutritional measures included changes in grassland management (N fertilization rate, stage of grass cutting) and in the level of concentrate feeding, and the replacement of grass silage by maize silage, beet pulp, straw or potatoes (Table 1; assumptions based on Reijs, 2007). Feed intake was calculated for an average Dutch dairy cow according to the Dutch system for feed intake capacity (Blok *et al.*, 2007; Zom *et al.*, 2012). Calculated N excretion strongly varied from 200 to 550 g N/day, and from 10 to 18 g N/kg fat and protein corrected milk (FPCM). Simulations show that rumen function plays a major role in this variation, by its effect on the extent of N digestion and site of N excretion (urine *v.* faeces) and by the occurrence of trade-offs such as an altered digestion and NE_L (Dijkstra *et al.*, 2011).

The $N:NE_L$ of concentrates can easily be reduced by a selection of N-poor feed ingredients. Practical feasibility of changes in the selection of feed ingredients mainly depends on limitations put in place by cost, targets for NE_L, by nutrient-specific critical levels for inclusion in the diet (e.g. dietary fat content), or other restrictions such as manure legislation (e.g. phosphorous content). Without exceeding any critical level of, for example, dietary fat content or rumen acidification potential, negative trade-off effects may remain limited in size. Nevertheless, there are some effects which may be of importance but do not become apparent from tables of feeding values, from feeding systems applied in practice, or from constraints and safety margins adopted with feed formulation.

For example, rumen starch digestion is subject to variation with the amount of starch fed. Variability in rumen digestion of a starch source means a variable contribution of that source to rumen microbial N synthesis, which is of importance to take into account when aiming for a minimum $N:NE_L$ and protein delivery still matching the cow's requirements. With an increase in dietary starch content and of starch intake from 4.0 to 6.5 kg/day, Oba and Allen (2003) observed an increase of rumen digestion of starch from 59% to 71% with high-moisture maize (absent with ground maize). The finding for high-moisture maize may reflect a higher amylolytic activity sustained in the rumen by a higher starch intake level. It cannot be explained from differences in DM intake (similar), fractional passage rate of rumen starch (higher), or the rumen starch pool (similar). Contrasting with this, with ground maize no such differences in rumen starch digestibility were found. In addition, Van Vuuren *et al.* (2010) did not observe an effect of maize starch intake (2.1 *v.* 6.2 kg/day) on rumen starch digestibility. Although *in situ* fractional starch degradability was numerically 25% lower on high starch intake, this effect seems to have been compensated by a numerically 23% lower fractional passage rate, explaining the similar rumen starch digestion at low and high starch intake (68% and 66%, resp.). In this case, a stimulatory effect of a high starch intake on the rumen pool size of amylolytic bacteria is not needed to explain observed results. Existence or absence of a trade-off of the level of starch intake probably has to be explained from the combination of factors of intrinsic degradation characteristics of starch,

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rumen retention time of starch and rumen pool sizes of starch and amylolytic bacteria.

Next to potential trade-offs of changes in the proportion of starch in the concentrate fraction of the diet, a change in the level of feed intake may cause trade-offs with respect to rumen and total tract digestion, thereby affecting the utilization of digested N by the cow. Roughage intake, chewing and rumination behaviour and rumen fermentation conditions can be affected and are relevant because of their influence on rumen passage rate, rumen content and activity of the amylolytic and fibrolytic microbiota (Dijkstra *et al*., 1992). A higher feed intake level reduces rumen, as well as faecal, fibre degradation. This is demonstrated in a classical study by Robinson *et al*. (1987), who combined measurements of the effect of an increase in starch content in concentrate (from 8% to 32% in concentrate; in effect from 5% to 21% in dietary DM) with an increase in DM intake (from 6 to 24 kg/day), of a diet consisting of one-third of hay and two-thirds of concentrate. Increasing dietary starch content decreased faecal NDF digestibility from 64% to 60% (from 58% to 53% for the rumen), compared with a decrease from 71% to 57% (from 65% to 49% for the rumen) with an increase of DM intake (average starch content). Part of the effect of an increase of dietary starch on NDF digestion may have been caused by a lower rumen pH (pH reduction from 6.6 to 6.0) because fibrolytic activity is generally assumed to become inhibited below pH values of 6.3 (Dijkstra *et al*., 1992). An even stronger effect of the dietary content of rapidly degradable starch was reported by Van Vuuren *et al*. (2010). With an increase in dietary starch from 12% to 33%, faecal NDF digestibility decreased from 84% to 75%, whereas there was no effect on rumen pH. Therefore, in contrast to the results of Robinson *et al*. (1987), rumen pH seems to have not been the reason for the decline in NDF digestibility. Still, *in situ* fractional degradation rate of NDF was decreased, reflecting a less available NDF source for fibrolytic bacteria with higher starch intake, and a lower rumen NDF digestion despite the slower passage from the rumen.

For roughages, both exchange of maize for grass, and altered grassland management (lower N fertilization rate, late cutting or postponed grazing for reduced N content) can strongly reduce N : NE_L. Such measures have important trade-offs with roughage intake, feeding value, milk production and N excreted per unit of milk. A lower N fertilization rate and extended cutting of grass will lower N : NE_L in grass, which can almost be halved when considering extremes (Bannink *et al*., 2010; Dijkstra *et al*., 2011). Simultaneously, this strategy may lower grass NE_L and result in a lower feed intake of grass. When compensated for with an increased concentrate allowance to maintain milk yield, a trade-off to take into account may be less favourable rumen fermentation conditions for fibrolytic bacteria, reducing roughage digestion (similar to what was discussed above for the effect of starch intake in the study of Robinson *et al*., 1987). Such a trade-off may partly off-set the reduction in N excretion per kg of milk achieved. Nevertheless, with grass as the main portion of a dairy cow diet, moving towards more extensive grassland management (less N fertilizer, later cutting), or to a partial exchange of grass for maize, will still have a profound effect on N intake and N excreted per kg FPCM (Dijkstra *et al*., 2011).

Quantifying N excretion and CH_4 emission

Enteric CH_4

Rate of enteric CH_4 emission is difficult to monitor on-farm and probably even more difficult to relate to the systematic effect of a specific nutritional measure taken by the farmer. So far, quantification of effects on enteric CH_4 relies on prediction models. Various models are available to quantify enteric CH_4, varying from the IPPC Tier 2 approach which assumes a constant 6.0% of gross energy intake being emitted as CH_4, recently updated to 6.5% (IPCC, 2006), to empirical models derived from large data bases by regression techniques (model evaluation and review by Ellis *et al*., 2008), to dynamic models which adopt a mechanistic approach by representing rumen fermentation processes and methanogenesis (e.g. Bannink *et al*., 2011). The IPCC Tier 2 method is commonly used in national inventory reports but it suffers from the problem that it assumes the proportion of ingested gross energy emitted as CH_4 is constant. The Tier 2 method hence only reflects the effects of DM intake level on CH_4 emission, neglecting all variation that is unrelated to DM intake; variation in the site of digestion of carbohydrates and protein, and the lack of fermentation of dietary fat. With the purpose to address the latter unexplained part of variation in enteric CH_4, modelling exercises have been conducted which delivered various quantitative models that diverge in the level of complexity represented in them, ranging from an empirical description of data sets with observations at the animal level (reviewed by Ellis *et al*., 2008), to the representation of the mechanisms of microbial activity and methanogenesis in the rumen (e.g. Dijkstra *et al*., 1992; Mills *et al*., 2001; Bannink *et al*., 2011; Figure 1). It remains open to discussion which of these models is best used for what purpose. In farm scale or regional monitoring, the choice often made is to use the Tier 2 approach or another empirical model to quantify enteric CH_4 (Schils *et al*., 2007), although there are exceptions to this (Beukes *et al*., 2011). A commonly used argument for empirical models or the Tier 2 approach to be the preferred choice is that these models would require a lower number and more easily accessible inputs. Evaluation of these empirical models adopted in farm-scale analyses against independent observations of enteric CH_4 is scarce or even absent. What has been reported seems to indicate their prediction capacity leaves room for improvement (Ellis *et al*., 2010). More complex models may serve in delivering more appropriate estimates of enteric CH_4 because they imply a logic with respect to the representation of mechanisms of rumen fermentation and occurrence of trade-offs at the rumen level (instead of the animal level) can be identified and quantified. Complex models might be considered to require too many

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Table 2 *Overview of the type of inputs required by various categories of prediction models relating feeding value and feed requirement to enteric methane, with input type categorized according to shade of grey*

Inputs required for model :	IPCC Tier 2	Regression	Dynamic
Digestibility / NE_L or ME value diet	✓	✓	
NE_L requirement → Feed intake	✓	(✓)	
Feed intake		(✓)	✓
Chemical composition → GE value diet	✓	(✓)	
Chemical composition		✓	✓
Rumen degradation characteristics			✓
Other (empirically available) dietary factors		(✓)	

A distinction is made between the Tier 2 approach according to IPCC methodology for performing inventories on greenhouse gas emissions (IPCC, 1997), empirical regression equations which include feeding value and a variety of other explanatory factors, and dynamic, mechanistic models which represent the mechanisms of the enteric fermentation process. The symbol (✓) indicates that this type of model input is used by a model category, whereas this symbol between brackets indicates the model input is conditional and depends on details of the model representation.

inputs, introducing uncertainty with each one of them, which adds to prediction error and also making them cumbersome to apply. Although indeed more inputs are required compared with empirical models, the presumption that these inputs in itself create more complexity cannot easily be made. A closer inspection of the type of inputs required by various types of models of enteric CH_4 makes clear that all model types make use of all input types (Table 2). Because it is needed for calculations on NE_L and feed requirement, all models relating feeding value to CH_4 make use of an input which relates to a digestibility characteristic of the feed. The use of more complex models suffers from a lack of data for the *in situ* rumen degradation characteristics, whereas data on NE_L are generally available (Bannink *et al.*, 2011). The more specific the case studied the more important these estimates become (e.g. Ellis *et al.*, 2012). All models somehow have to address chemical composition of the diet and prediction equations are available for NE_L, but so far equations are lacking for the estimation of *in situ* degradation characteristics. However, even with prediction equations available for estimation of NE_L, the error of estimation may still be substantial. Further, the full list of inputs required by a complex model such as that used by Bannink *et al.* (2011) or Beukes *et al.* (2011) should not be considered an indication of higher complexity of these inputs compared with those needed by empirical models. Several dietary chemical fractions are complementary to each other simplifying their estimation, by using generic or table values, reducing the number of uncertain inputs. From this respect, the input requirements of the various quantitative modelling approaches is not so fundamentally different as it appears from the type and number of equations involved in the mathematical description of more complex models.

The differences between modelling approaches strongly relates to the origin of inputs chosen to be used (at the diet or ingredient level, at the animal and digestive level, or at the rumen fermentation level) and how these are handled by the model. The model of choice for quantifying CH_4, hence depends on the origin of data, the specific aim(s) the model can be used for, and the level of detail required to answer the questions posed. If the aim is to have a detailed insight in the effects of nutrition on enteric fermentation and its consequences for CH_4 emission next to diet digestibility, N excretion and cow productivity, rumen details matter. Empirical models cannot be used to deliver details on the rumen or even the digestive level, with the Tier 2 approach perhaps being least applicable in this respect because it only generates a generic value for CH_4 emission based on gross energy intake. It is not very specific which is in contrast to the aim to establish differences between farm management options directed at lowering CH_4 emissions.

N excretion

Quantifying the details of N digestion and N metabolism, and the variation in N and urea flows in a dairy cow is complex. Similar to what was discussed for CH_4, the rumen also plays an important role in the extent and site of N digestion, in microbial protein synthesis (the main digestible protein source for a dairy cow) and in recycling of blood urea to the rumen (Figure 2a). As a result, variation in rumen function contributes to variation in the partitioning of excretion of ingested, non-utilized N in urine and faeces. In contrast to quantifying CH_4,

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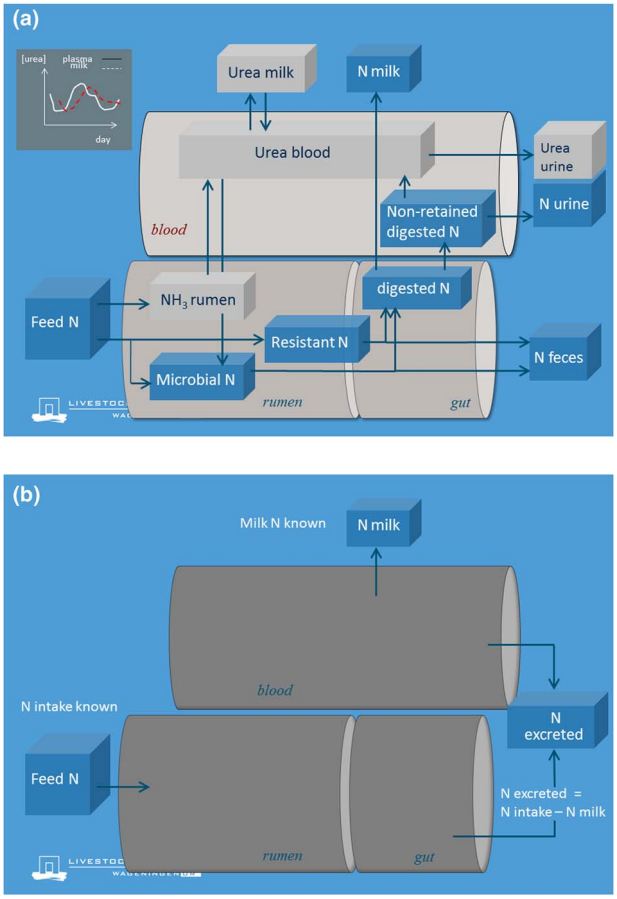


Figure 2 Nitrogen and urea flows in the dairy cow (a) and the approximation of N excretion by a nitrogen balance calculation (b).

quantifying N excretion has the advantage that actual observations are available on N flows at the animal level, viz., feed intake, feed requirement, diet composition and milk protein yield. Under assumption of zero N balance, these observations allow a quick estimate of N excreted (N excreted equal to N intake minus N milk; Figure 2b). Assumption of a zero N balance will bias estimated N excretion but the size of error remains small, particularly when long periods are considered (e.g. whole lactation). For specific relatively short periods of time (e.g. first weeks after calving, late pregnancy) bias may be significant however, and lead to under- or overprediction of N excretion in the order of 50 g N/day (Van Knegsel *et al.*, 2007), which may exceed 10% of estimated N excretion. Bias can partly be prevented by taking into account *a priori* the physiological state of the cow and make assumptions on N balance. But, in combination with uncertainty about the estimates on feed intake and dietary composition (generally no observations available), error may remain substantial when estimating momentary N excretion for certain stages in the lactation cycle.

Most variation in N excretion is because of variation in urea excretion in urine. Therefore, it is most important to quantify this most variable part of N excretion. Milk urea content reflects urea concentration in blood, and hence is an indicator of urea flow through blood to urine (Figure 2a). Blood

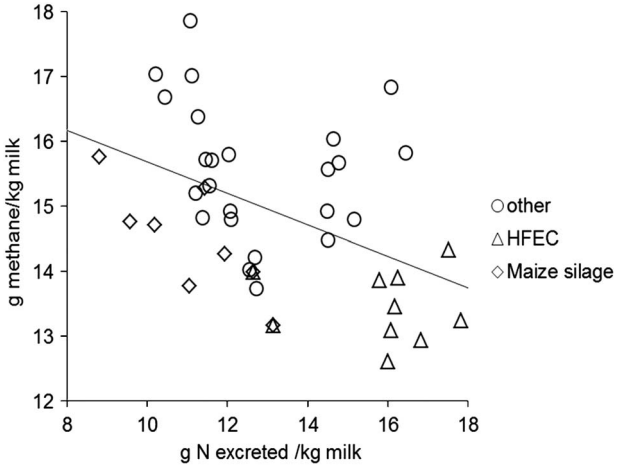


Figure 3 Methane emission *v.* N excretion per kg fat and protein corrected milk for 40 simulated diets. Effects were simulated of a changed grassland management (350 *v.* 150 kg N artificial fertilizer/ha next to 70 kg N/ha from animal manure for first cut; early at 3000 kg/ha *v.* late cutting at 4500 kg/ha), changing level of concentrate allowance (20% or 40% of DM intake), and exchange of grass silage for various other ingredients (maize silage, beet pulp, potatoes, straw). Separate symbols are used to indicate the low methane emissions simulated for diets with grass silage exchanged for maize silage (Maize silage, ◇), and for diets including high N-fertilization and early-cutting (HFEC; Δ), compared with the remainder of diets simulated (Other; ○).

urea levels are the net result of the amount of urea produced, urea excreted by the kidneys and urea recycled to the gastrointestinal tract (Reynolds and Kristensen, 2008). Although, milk urea appears closely associated with total excretion rate of N and urea in urine, over a wide range of values at least, some dynamical aspects of the aforementioned processes are reflected in blood and milk urea concentrations (Spek *et al.*, 2013). The principal concern remains that a concentration (milk urea content) is used as an approximation of urea flux (urea excretion). Accuracy of calculating N excretion from milk urea will be discussed further in the section related to on-farm indicators.

Interactions between N excreted and CH_4 emitted

Because rumen function has an important influence on feed digestion, and associated N excretion and enteric CH_4 emission per kg FPCM, investigation of the effect of nutritional measures on emissions requires that details on rumen fermentation mechanisms are taken into account. This can be achieved by making use of a dynamic, mechanistic model of fermentation and digestive processes in the gastrointestinal tract of a cow. Such a model (Dijkstra *et al.*, 1992; Mills *et al.*, 2001; Bannink *et al.*, 2006 and 2008) is in use as a Tier 3 approach in the Dutch inventory of greenhouse gas emissions in dairy cattle as described by Bannink *et al.* (2011). Recently, Dijkstra *et al.* (2011) used the model to study a variety of N reducing nutritional measures on enteric CH_4, as well as N excretion, by performing simulations for 40 different diets, including two extremes in N fertilization of grassland as well as in sward weight at cutting, two levels of concentrate

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allowance and various exchanges of grass silage (Table 1). The results indicated a moderate ($r^2 = 0.22$; Figure 3) but negative trend (slope of −0.24) for g CH_4/kg FPCM emitted per g N excreted/kg FPCM (Dijkstra *et al.*, 2011). The results indicate that there is a large variation in CH_4 emission for the various N reducing nutritional measures. In some cases, there were synergistic effects (Figure 3; more maize silage at the expense of grass silage), in other cases there was a trade-off of an increased N excretion with reduced CH_4 emission (Figure 3; high N fertilization and early cutting of grass). Recently, such a negative trend with much variation remaining was confirmed in a simulation study specifically oriented on trials performed on high-sugar grasses (Ellis *et al.*, 2012). Although *in vivo* observations in well-designed experiments remain sparse, there is some support of a decrease in N excretion via grassland management resulting in an increase in CH_4 emissions. Results of lactating cows fed grass herbage in climate controlled respiration chambers, used for model evaluation by Bannink *et al.* (2010), as well as results of Murray *et al.* (2001) of sheep kept in tunnels on pasture suggest that with an extreme increase of N content in grass (2.5% to 4.5% of DM) or in N fertilization rate (70 *v.* 270 kg N/ha), respectively, CH_4 emission per kg DM may be up to 20% lower. More *in vivo* results from well-designed experiments are needed however for more conclusive results.

Therefore, nutritional measures to reduce N excretion and related N emissions do not necessarily reduce greenhouses gas emission (in CO_2 equivalents). According to the general trend that was simulated by Dijkstra *et al.* (2011), 1 g reduction in N excretion/ kg FPCM increases CH_4 emission by 0.24 g/kg milk. Suppose only 1% of excreted N becomes emitted as N_2O, and the ratio of global warming potential of N_2O to that of CH_4 is 298 : 25, then the reduced N_2O emission is more or less compensated by an increased enteric CH_4 emission. The large variation in simulation results for N excretion and CH_4 emission makes clear that generic values better not be used when aiming to analyse specific farming conditions, farm types or nutritional measures. Generic values do not hold when research questions are very specific because details on rumen fermentation processes and digestive function matter when aiming to evaluate the effects on enteric CH_4 next to effects on N excretion. Farm N surplus has been mentioned as an indicator of the level of greenhouse gas emissions from farms (Schils *et al.*, 2007). However, the present results indicate that this does not necessarily hold. It may hold when coming downwards from extremely high farm N surpluses, with high N_2O emissions and with N_2O as the predominant source of greenhouse gas. However, this is likely not a good reference for current practice and certainly not with the situation under a stringent manure legislation (e.g. the EU nitrate and phosphate directives). Moreover, farm type and details on farm management have a profound impact on which nutritional measures actually are feasible and on how they will affect enteric CH_4 and N excretion realized per kg FPCM. This impact will be hard to evaluate when non-specific values or average values from inventory studies are adopted.

On-farm indicators for quantification of N excreted and CH_4 emitted

In previous sections, the methods to quantify N excretion and enteric CH_4 were discussed. Although different models may be adopted, they are all prone to error and uncertainty related to the model inputs. An alternative to the uncertainty associated with model calculations would be on-farm measurement. However, it is difficult to measure rates of CH_4 emission or N excretion as such. Measurements which are feasible are normally those of concentrations of compounds in samples of, for example, air (ppm CH_4) or manure (g N/kg DM), whereas estimates of rates are needed (g CH_4/day; g N/day). Even when samples are taken on a frequent basis, interpretation of them as a rate relies on the (measurement and calculation) method to transfer measured concentration in samples into estimates of daily quantities or fluxes (e.g. Garnsworthy *et al.*, 2012). Because of technological issues, the need for technology assessment, the operational costs involved, and the continuous variation in feeding conditions and sampling in practice, a pragmatic and straightforward alternative to the technology of air and manure sampling would be the sampling of milk which is already in place in current practice. The rate of milk production (kg/day) is known for every individual animal, concentrations of milk urea are associated with N excretion rate as discussed earlier, and milk fat composition is associated to rumen fermentation processes and hence CH_4 emission. The association of both N excretion and CH_4 emission to milk composition exists (Dijkstra *et al.*, 2011; Spek *et al.*, 2012). However, reliable prediction equations or quantitative models are needed to let them be a realistic alternative for continuous on-farm measurement, and to let them become useful to monitor short-term developments in CH_4 emission and N-excretion and control these by nutritional means.

Milk urea seems to be a useful indicator when considering the whole range of N excretion, but it clearly is an inaccurate indicator when considering the far more narrow operational working range of milk urea for a farmer (Figure 4a). A large part of variation in milk urea content is unrelated to N excretion and must be related to several dietary, animal and management factors as recently reviewed by Spek *et al.* (2013). The existence and impact of such factors on milk urea is nicely illustrated by the fact that heritability of milk urea content appears to be not even slightly related to efficiency of N utilization as determined from observations of 723 cows in 26 trials on feed intake, milk production and cow weight (Šebek *et al.*, 2007). This finding indicates there was no causal relationship between heritability of milk urea content and efficiency of N utilization (and hence N excretion). Other factors must be involved with heritability of milk urea content. Likely, similar factors first need to be understood, and subsequently to be sifted out, in order to let milk urea become an accurate indicator of N excretion and be a useful tool for the farmer to optimize feeding strategy, minimize N excretion and identify differences between individual cows. For the moment, perhaps it is most

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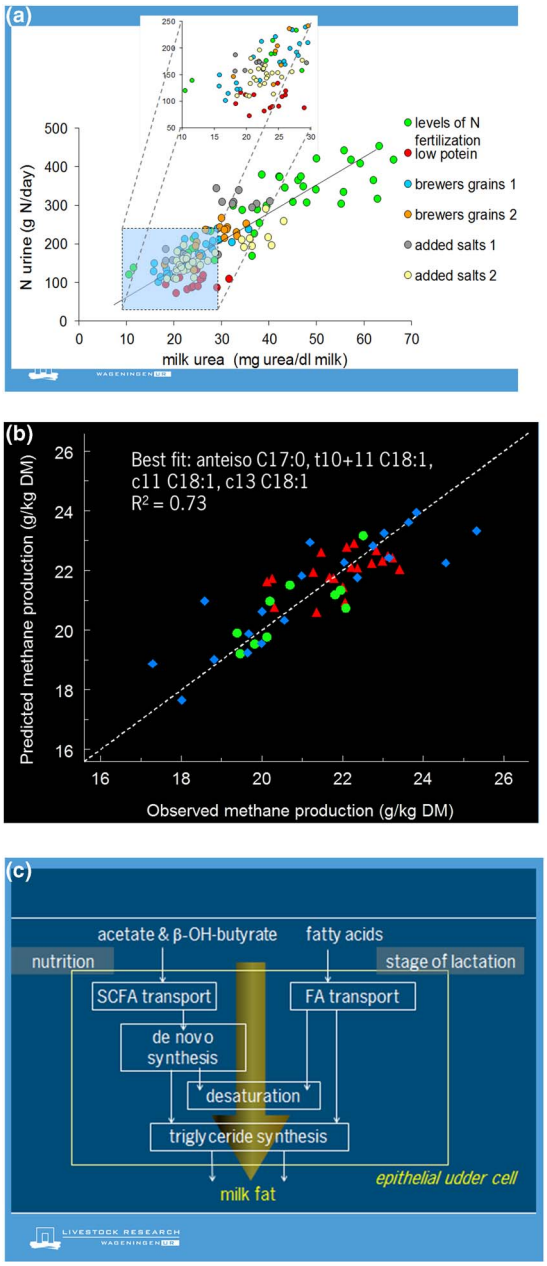


Figure 4 Estimating (a) N excretion from milk urea content (dataset described by Schröder *et al.*, 2005), (b) enteric CH_4 emission from the profile of fatty acids in milk fat as an indicator by an empirical method (derived from Dijkstra *et al.*, 2011), (c) milk fat composition by a mechanistic approach describing regulatory mechanism involved in milk fat synthesis in udder cells.

accurate simply to estimate N excretion by a N balance calculation approach.

Comparable to milk urea being related to N excretion, milk fat composition is likely susceptible to variation unrelated to rumen fermentation and enteric CH_4 (Vlaeminck *et al.*, 2006). Associations between milk fat composition and enteric CH_4 have been documented (e.g. Dijkstra *et al.*, 2011). However, similar to the case discussed for milk urea (Figure 4a), with an R^2 of 0.73 for the equation for the whole range of CH_4 emission observed (Figure 4b), to predict accurately enough in the range of interest of the farmer the explanation of observed variation has to improve. Various factors are involved in the regulation of milk fat composition. More mechanistic modelling approaches that attempt to address the underlying regulatory mechanisms of milk fat synthesis may assist the more empirical approaches that attempt to describe the variation in milk fat composition related to enteric CH_4 (Figure 4c). This may include a representation of the effect of diet (Bauman *et al.*, 2011), physiology (Kelsey *et al.*, 2003) and genotype (Schennink *et al.*, 2007; Mach *et al.*, 2012) on milk fatty acid profile. For the development of milk fat composition as an on-farm indicator of enteric CH_4 in any case requires large data sets which include accurate *in vivo* measurement of CH_4 emission. At this moment, an indicator of enteric CH_4 is unavailable and estimation of CH_4 emission relies on the use of quantitative models. The model of choice depends on model accuracy and on the preferred level of detail in the model representation (i.e. of the underlying mechanisms and trade-offs in CH_4 emission with N reducing measures).

Conclusions

Rumen function has a profound impact on the extent and site of feed digestion, on the extent and site of N excretion and on enteric CH_4 emission. Simulation studies indicate that the direction and the extent of changes in enteric CH_4 depend on the approach taken to reduce N excretion. Results indicate changes in N excretion must be expected to be accompanied by changes in enteric CH_4 emission, and that simultaneous changes may fully compensate each other with respect to their contribution to greenhouse gas emission. When the aim is to become precise in accounting for such trade-offs, details on rumen fermentation mechanisms matter and need to be addressed, or it should at least be made clear why such details can be ignored. Before milk analysis can be used as an indicator for N excretion and enteric CH_4 emission accuracy of these measures need to be improved. Development of these indicators require modelling efforts which preferably include logic and rational before they are applied as a proxy for the mechanisms responsible for N excretion and CH_4 emission. Until such indicators are available, estimates of N excretion and enteric CH_4 emission on farms are better off being based on extant quantitative models that make use of information available on-farm regarding animal performance and dietary characteristics.

Further information

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The Dairy Solutions Symposium is a biennial event that covers a wide variety of themes and topics of relevance and importance to the dairy industry. The aim is to provide high level, up-to-date

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information and research to dairy professionals, technologists and scientists. In 2012, the theme addressed the biggest challenge facing all those involved in dairy production: optimizing production efficiency while lowering environmental impact. For more information, please visit www.dairycowsolutions.com or contact dairycowsolutions@alltech.com.

References

Bannink A, Van Schijndel MW and Dijkstra J 2011. A model of enteric fermentation in dairy cows to estimate methane emission for the Dutch National Inventory Report using the IPCC Tier 3 approach. Animal Feed Science and Technology 166, 603–618.

Bannink A, Kogut J, Dijkstra J, France J, Kebreab E, Van Vuuren AM and Tamminga S 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating dairy cows. Journal of Theoretical Biology 238, 36–51.

Bannink A, France J, Lopez S, Gerrits WJJ, Kebreab E, Tamminga S and Dijkstra J 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Animal Feed Science and Technology 143, 3–26.

Bannink A, Smits MCJ, Kebreab E, Mills JAN, Ellis JL, Klop A, France J and Dijkstra J 2010. Simulating the effects of grassland management and grass ensiling on methane emission from lactating cows. Journal of Agricultural Science 148, 55–72.

Bauman DE, Harvatine KJ and Lock AL 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annual Review of Nutrition 31, 299–319.

Beukes PC, Gregorini P and Romera AJ 2011. Estimating greenhouse gas emissions from New Zealand dairy systems using a mechanistic whole farm model and inventory methodology. Animal Feed Science and Technology 166–167, 708–720.

Blok MC, André G, Brandsma GG, Van Straalen WM and Van Duinkerken G 2007. Voeropnamemodel Melkvee, versie 2007. CVB-documentatierapport 51, CVB, Productschap Diervoeder, NL.

Cant JP 2005. Integration of data in feed evaluation systems. In Quantitative aspects of ruminant digestion and metabolism, 2nd edition (ed. J Dijkstra, JM Forbes and J France), pp. 707–725. CAB International, Wallingford, UK.

Dijkstra J, Oenema O and Bannink A 2011. Dietary strategies to reducing N excretion from cattle: implications for methane emissions. Current Opinion in Environmental Sustainability 3, 414–422.

Dijkstra J, Neal HD, Beever DE and France J 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. Journal of Nutrition 122, 2239–2256.

Ellis JL, Bannink A, France J, Kebreab E and Dijkstra J 2010. Evaluation of enteric methane prediction equations for dairy cows used in whole farm models. Global Change Biology 16, 3246–3256.

Ellis JL, Dijkstra J, Kebreab E, Bannink A, Odongo NE, McBride BW and France J 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. Journal of Agricultural Science 146, 213–233.

Ellis JL, Dijkstra J, France J, Parsons AJ, Edwards GR, Rasmussen S and Kebreab E 2012. Effect of high-sugar grasses on methane emissions simulated using a dynamic model. Journal of Dairy Science 95, 272–285.

Garnsworthy PC, Craigon J, Hernandez-Medrano JH and Saunders N 2012. Variation among individual dairy cows in methane measurements made on farm during milking. Journal of Dairy Science 95, 3181–3189.

IPCC 2006. IPCC guidelines for national greenhouse gas inventories. Intergovernmental. Panel on Climate Change, IGES, Japan.

Janssen PH 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Animal Feed Science and Technology 160, 1–22.

Johnson KA and Johnson DE 1995. Methane emission in cattle. Journal of Animal Science 89, 1302–1310.

Kelsey JA, Corl BA, Collier RJ and Bauman DE 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. Journal of Dairy Science 86, 2588–2597.

Mach N, Blum Y, Bannink A, Causeur D, Houee-Bigot M, Lagarrigue S and Smits MA 2012. Pleiotropic effects of polymorphism of the gene diacylglycerol-O-transferase 1 (DGAT1) in the mammary gland tissue of dairy cows. Journal of Dairy Science 95, 4989–5000.

Mills JN, Dijkstra J, Bannink A, Cammell B, Kebreab E and France J 2001. A mechanistic model of whole-tract digestion and metahnogenesis in the lactating dairy cow: model development, evaluation, and application. Journal of Animal Science 79, 1584–1597.

Murray PJ, Gill E, Balsdon SL and Jarvis SC 2001. A comparison of methane emissions from sheep grazing pastures with differing management intensities. Nutrient Cycling in Agroecosystems 60, 93–97.

Oba M and Allen MS 2003. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. Journal of Dairy Science 86, 184–194.

Reijs JW 2007. Improving slurry by diet adjustments: a novelty to reduce N losses from grassland based dairy farms. PhD Thesis, Wageningen University, The Netherlands, 203pp.

Reynolds CK and Kristensen NB 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. Journal of Animal Science 86, 293–305.

Robinson PH, Tamminga S and Van Vuuren AM 1987. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. Livestock Production Science 17, 37–62.

Schennink A, Stoop WM, Visker MH, Heck JM, Bovenhuis H, Van der Poel JJ, Van Valenberg HJ and Van Arendonk JA 2007. DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. Animal Genetics 38, 467–473.

Schils RLM, Olesen JE, Del Prado A and Soussana JF 2007. A review of farm level modelling approaches for mitigating greenhouse gas emissions from ruminant livestock systems. Livestock Science 112, 240–251.

Schröder JJ, Bannink A and Kohn RA 2005. Improving the efficiency of nutrient use on cattle operations. In Nitrogen and phosphorus nutrition in cattle (ed. E Pfeffer and A Hristov), pp. 255–279. CAB International, Wallingford, UK.

Šebek LBJ, Van Riel J and De Jong G 2007. The breeding value for milk urea as predictor for the efficiency of protein utilization in dairy cows. Animal Sciences Group, Report 81. Lelystad, The Netherlands.

Spek JW, Dijkstra J, Van Duinkerken G and Bannink A 2013. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. Journal of Agricultural Science 151, 407–423.

Spek JW, Bannink A, Gort G, Hendriks WH and Dijkstra J 2012. Effect of sodium chloride intake on urine volume, urinary urea excretion, and milk urea concentration in lactating dairy cattle. Journal of Dairy Science 95, 7288–7298.

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M and de Haan C 2006. Livestock's long shadow: environmental issues and options. FAO, Rome.

Van Knegsel AT, Van den Brand H, Dijkstra J, Van Straalen WM, Heetkamp MJ, Tamminga S and Kemp B 2007. Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. Journal of Dairy Science 90, 1467–1476.

Van Vuuren AM, Hindle VA, Klop A and Cone JW 2010. Effect of maize starch concentration in the diet on starch and cell wall digestion in the dairy cow. Journal of Animal Physiology and Animal Nutrition 94, 319–329.

Van Zijderveld SM, Gerrits WJJ, Dijkstra J, Newbold JR, Hulshof RBA and Perdok HB 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. Journal of Dairy Science 94, 4028–4038.

Vlaeminck B, Fievez V, Tamminga S, Dewhurst RJ, Van Vuuren AM, DeBrabander D and Demeyer D 2006. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern. Journal of Dairy Science 89, 3954–3964.

Zom RLG, André G and Van Vuuren AM 2012. Development of a model for the prediction of feed intake by dairy cows: 1. Prediction of feed intake. Livestock Science 143, 43–57.

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