C1OXO902- Systems analysis to quantify the role of farm management in GHG emissions and sinks for pastoral sectors

Milestone: 1.2.1 International review of literature and data bases

Objective: Re visit a previous internal report (Romera et al., 2008, PGGRC) on models available for testing against calorimeter data.

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Title: Models available for predicting methane emissions from grazing cattle: An overview

Summary:

Models of methanogenesis were classified in three groups: statistical, semi-mechanistic and dynamic and mechanistic models:

- *Statistical models* have been evaluated over the last 30 years, showing a moderately good behaviour. Most of the evaluations showed the model of Moe and Tyrell (1979), still, as the best one. Although statistical models appear useful for quick appraisals of the level of CH_4 emission, particularly when information on dietary ingredients, production conditions and feed intake level is lacking; they do not explain the variation in CH_4 emission for differing feeding regimens sufficiently accurately. This issue is intimately related to their empirical nature, and to cope with it statistical models need to be built up on a base of broader and more heterogeneous data base.
- S*emi-mechanistic models*. In an attempt to keep simplicity of prediction and gather some benefit of a mechanistic approach, non-linear semi-mechanistic models [i.e. Mills et al. (2003) and Volden (2010)] are proposed as a step forward alternative to statistical models. This approach, however, warrants development and parameterization to pastoral conditions.
- *Dynamic and mechanistic models* are more suitable for predicting CH_4 emission from ruminants, what is the result of the mechanistic nature of its construction and description of rumen digestive processes. Three major rumen/cow models recognized in the literature: Molly, Anje and Karoline. Although these models behave better than statistical models, unfortunately, and despite of their mechanistic framework, the equations used to predict CH_4 production are empirical. Moreover, these particular empirical equations (stoichiometric models for VFA predictions) were developed using a still narrow data set, which yet constraints the full/ broad context

flexibility and applicability for what mechanistic models were designed, at least in regards to enteric CH_4 emissions. A mechanistic representation of rumen fermentation patterns in proposed then to improve accuracy and sensitivity of this type of models.

Principles of thermodynamic have been suggested for developing dynamic and mechanistic models of the rumen. Although the thermodynamic approach of modelling rumen fermentation pattern and then CH_4 is promising, it is still in early stages. Even if thermodynamic principles were ruling fermentation processes of the rumen/ cow models, they would represent a validation challenge, since there is almost none data in this regard, and even less proper experiments set for this purpose. Consequently, this approach warrant further research and model development.

Scaling-up. Giving attention to scales is important, since simple scaling-up or down leads to errors in phenomena interpretation. Thus it is suggested the incorporation of dynamic and mechanistic models of rumen and even better whole cows to '*whole farm models*' to progress in the understanding and assessment of CH_4 at a larger scale than the rumen.

INTRODUCTION

Greenhouse gases are atmospheric gases that absorb and then re-emit long-wave radiation released by the earth back to the earth surface (Clark and Eckard, 2010). Over the last century, carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), the main anthropogenic green house gases, have markedly increased as result of human activity (Clark and Eckard, 2010). Methane, for example, has doubled its contribution to the greenhouse gases, becoming the major contributor of agricultural related greenhouse gases. Globally, the ruminant-livestock sector produces 17-30% of anthropogenic induced CH_4 emissions (Ellis et al. 2010). Due to the main source of CH_4 is the enteric fermentation of ruminants, reduction of CH_4 emissions in countries with high stocking rate of ruminant livestock presents a major challenge. Consequently, the research interest in reducing CH_4 emissions from ruminants has exponentially increased.

'Silver bullet' – like approaches (e.g. dietary additives, rumen defaunation or inmunization) have shown partial success in reducing the enteric CH_4 emissions from ruminants (Yan et al., 2010). The 'partial success (i.e. variable results)' of these strategies relates to their inconsistency and lack of practical demonstration of their benefits at farm system context (Yan et al., 2010; Clark and Eckard, 2010). At this level of aggregation, the large variations in CH_4 emissions have been attributed to feeding regimens, and so to dietary factors (Ellis et al., 2007; Yan, 2009), what have led most of the current experimental research and modelling approaches efforts. Although the dietary/ feeding management approach seems to be more consistent ('higher partial successes', i.e. less variable results), emission of enteric CH_4 greatly vary between type of animal (i.e. cattle, sheep, and deer), within type of animal (even under the same diet) and within individual animal. Within individual animal variation may relate to nutrional-physiological state; while within type of animal might reflect a potential effect of genetic merit on enteric CH_4 emissions yields as an hypothetical differential capacity of energy partitioning (Yan et al., 2010). However, there is virtually no information about the two latter issues.

The complexity of CH_4 production and its evident multiple sources of variations, therefore, requires a systemic approach of gathering, analyzing and interpreting available information, as well as cost-effective tools helping to draw new lines of specific research from small (rumen) to larger (farm-region) spatio-temporal scales. Mathematical models offer the potential to describe scenarios of complex interactions and evaluate hypothetical and practical intervention strategies for any given situation, thereby providing a low cost and quick estimate of best management practices. Under a general objective of improving systems analysis to quantify the role of farm management in greenhouse gases emissions and sinks for pastoral farming, the present work focused on the description and conceptual evaluations of current simulation models for estimating enteric CH_4 emissions from ruminants.

MODEL TYPES

Models of methanogenesis process (as any other process) can be classified in two groups: statistical models and dynamic and mechanistic models. Statistical models are constructed from the data, and have been used as a tool to describe empirical relationships between particular input and outputs of a system. Statistical models treat the system as a 'black box', not focusing on the underlying processes (Thornley, 1998). Mechanistic models are based on assumptions about the mechanisms of processes represented in the model, which are thought to be important in a particular system. There are advantages and disadvantages for each type of model; the choice depends entirely on the purpose. Particular considerations and examples of each type of model, used in this case for predicting CH_4 emissions, are presented below.

Statistical models.

Empirical relationships used to predict enteric CH_4 yield and production have been around for 80 years (Kriss, 1930). From the pioneer work of Kriss, 1930, several other statistical models have been developed (and published) aiming to obtain a rapid and simple estimation of enteric CH_4 at the time of assessing CH_4 emissions at larger scales. These models, in fact were and are being used to obtain values for inventory purposes (See Ellis et al., 2010). As mentioned by Mills (2008), and then corroborated by Ellis et al. (2010), Wilkerson et al. (1995) summarized the most relevant statistical models of methanogenesis. These and other models are presented in Table 1.

Table 1. Statistical models to predict enteric CH_4 emissions. Adapted from †Wilkerson et al., (1995) (unit: Mcal/d) and ‡Ellis et al. (2010) (unit: g/d)

Reference	Model/s
†Kriss (1930)	(18 +22.5 × DMI (kg/d) × 0.013184 (Mcal/g of CH_4)
†Bratzler and Forbes (1940)	(17.68 + 0.04012 × digested carbohydrate (g/d)) × 0.013184 (Mcal/g of CH_4)
†Axelsson (1949)	-0.494 + 0.629 × DMI (kg/d) – 0.025 × DMI^2 (kg/d)
†Blaxter and Clapperton (1965)	(1.30 + 0.112 × energy digestibility determined at maintenance intake (% of gross energy) + multiple of maintenance × (2.37 – 0.050 × energy digestibility at maintenance intake (%of gross energy))) + 100 × gross energy intake (Mcal/d)
†Moe and Tyrrel (1979)	**Intake of carbohydrate fractions** 0.814 + 0.122 × nonfiber carbohydrate (kg/d) + 0.415 × hemicellulose (kg/d) + 0.633 × cellulose (kg/d) **Intake of digested carbohydrate fractions** 0.439 + 0.273 × digested nonfiber carbohydrates (kg/d) + 0.512 × digested

	hemicellulose (kg/d) + 1.393 × digested cellulose (kg/d)
†Holter and Young (1992)	**Non lactating cows** (12.12 – 0.00542 × BW (kg) – 0.0900 × ADF (%DMI) + 0.1213 × ADF digestibility (%) – 2.472 × digestible energy (Mcal/kg DM) + 0.0417 × NDS digestibility (%) – 0.0748 × cellulose digestibility (%) + 0.0339 × hemicellulose digestibility (%)) + 100 × gross energy intake (Mcal/d) **Lactating cows fed supplemental dietary fats** 2.898 – 0.0631 × milk (kd/d) + 0.297 × milk fat (%) – 1.587 × milk protein (%) + 0.0891 × CP (5DM) + 0.1010 × forage ADF (% DM) + 0.102 × (DMI (kg/d) – 0.131 × ether extract (% DM) + 0.116 × DM digestibility (%) – 0.737 × CP digestibility (%)) + 100 gross energy intake (Mcal/d) **Lactating cows fed supplemental dietary fats** (2.927 – 0.0405 × milk (kg/d) + 0.335 × milk fat (%) - 1.225 × milk protein (%) + 0.248 × CP (% DM) - 0.448 × ADF (% DM) + 0.502 × forage ADF (% DM) + 0.0352 × ADF digestibility (%)) + 100 × gross energy intake (Mcal/d)
‡Kirchgeßner et al. (1995, eqn 1)	63 +79 ×crude fiber (kg/d) +10 ×nitrogen free extract (kg/d) +26 ×crude protein (kg/d) - 212 ×fat (kg/d)
‡Kirchgeßner et al. (1995, eqn 2)*	10 +4.9 ×milk yield (kg/d) +1.5 × liveweight ($kg^{0.75}$) **Intercept start at 59 if the diet is based on maize silage*
‡IPCC (1997) Tier II	[0.06 × gross energy intake (Mj/d)]/0.05565
‡Corre (2002)	[50 + 0.01 × milk yield (kg/d) × 365]/365 × 1000
‡Giger-Reverding et al. (2003)	[45 – 0.018 ×dry mater intake $(\text{g/kg liveweight/d})^2$ – 1.84 × linolenic acid (% DM) – 84.2 × fatty acids with a chain length equal or greater than 20 carbon atoms (% DM)] × dry mater intake (kg/d) × 0.6802
‡Schils et al. (2006)	20 × concentrate intake (kg as fed/d) + 22 × maize silage intake (kg DM /d) + 27 × grass intake (kg DM/d)

According to Wilkerson et al. (1995), all the equations were adequate to predict methane production from non-lactating animals. However, the one from Blaxter and Clapperton (1965) had the highest concordance correlation coefficient for non-lactating animals. For lactating animals, however, the behaviour of the models were variable and the one that performed the best was equation of Moe and Tyrrell (1979) using the intake of carbohydrates fractions. Palliser and Woodward (2002), using grass herbage-based diets, reported the same as Wilkerson et al. (1995) when comparing the statistical models of Moe and Tyrrell (1979), Blaxter and Clapperton (1965) and Kirchgeßner et al. (1995). Using more refined data set, thirty one years later, Ellis et al. (2010) arrived at similar conclusion, indicating that the Moe and Tyrrell (1979) model performed the best. Ellis et al. (2010) compared the performance of several empirical models for methane prediction for dairy cows used in some whole farm models. The models compared were: Moe and Tyrrell (1979), Blaxter and Clapperton (1965), Corre (2002), Giger-Reverding et al. (2003), IPCC-Tier I (1997), IPCC-Tier II (1977), Kirchgeßner et al. (1995, eqn. (1 and 2)) and Schils et al. (2006).

Tamminga et al., (2007) evaluated 22 different models ranging from simple static (most of them mentioned and described before) to complicated dynamic mechanistic models in terms of their ability to accurately determine cattle methane emission from various feeding strategies. The authors grouped the models into three categories: static empirical, dynamic empirical and dynamic mechanistic models (See next section). The static empirical performed better than dynamic empirical and dynamic mechanistic models in some circumstances, but not in others. The authors concluded that statistical models did not explain the variation in CH_4 emission with sufficient accuracy for differing nutritional treatments. According to Tamminga et al. 2007) the 'failure' of the statistical models was intimately related to their empirical nature. In the same line of Tamminga et al. (2007), Kebreab et al. (2006) evaluated the capability of models to predict CH_4 emission from ruminants. Kebreab, et al. (2006) tested six models; the linear model of Moe and Tyrell (Moe and Tyrell 1979), two empirical models proposed by Mills et al. (2003), the dynamic model of Kebreab et al.

(2004) and Tier 1 and Tier II models recommended by IPCC (IPCC 1996). Essentially, the conclusions drawn by these authors are the same as Tamminga et al. (2007), in the sense that a full assessment of mitigation options requires mechanistic models. Nevertheless, static models appear useful for a quick appraisal of the size of changes in the level of CH_4 emission in ruminants that may be expected with changes in management or nutrition, in particular when information on dietary ingredients, production conditions and feed intake level is lacking (Tamminga et al., 2007).

Unfortunately, most of the statistical models were built on the basis of North American and /or European data (animals end diets), which in some occasions it may certainly limit their application to grass herbage-based diets, particularly in grazing feeding scenarios, as supported by the differences reported between ‘in-door’ feeding and grazing by Pinares-Patiño and Clark (2010).

Mills (2008) pointed out that another risk of an empirical approach is to assume nonexistent (biologically based) relationships (cause and effect) with ‘only the aim of getting better correlations’. An example of these type of ‘errors’ is shown in the model of Holter and Young (1992) (Mills, 2008). The model of Holter and Young (1992) implies a significant effect of milk yield and milk composition on CH_4. Milk yield and its composition are function of nutrition and DMI. Therefore, according to Mills (2008) the implications of this model could be misleading. These considerations, however, do not have to stop the use and/ or consideration of factors related to milk. For example, Yan et al. (2010) propose a simple statistical model developed on the bases of calorimetric data obtained from 20 studies (mainly using either fresh grass or grass silage), considering gross energy intake or energy milk outputs.

$$CH_4 - E/ GEI = -0.0256 \times (E_{l(0)}/ MBW) + 0.075$$

Where,

$CH_4 - E$ = Methane energy output (Mj/ d)

GEI = Gross energy intake

$E_{l(0)}$ = Milk energy output adjusted to zero energy balance(Mj/d)

$E_{l(0)} = E_l + a \times$ energy balance

Where,

E_l = milk energy output

a = 0.95 and -0.84 for positive and negative energy balance, respectively (AFRC, 1990).

MBW = Metabolic body weight ($BW^{0.75}$)

Yan et al. (2010) also showed that CH_4 output is negatively related to energy metabolizability and the efficiency of utilization of ME for lactation (see model below and Figures 1 and 2 from Yan et al. 2010). Therefore, selection for more efficient cows in using energy would offer an effective approach to reduce CH_4 emissions.

$$CH_4 - E/ E_{l(0)} = -9.418 \times (E_{l(0)}/ MEI) + 10.824 \times (E_{l(0)}/ MEI)^2 + 2.193$$

Where,

$CH_4 - E$ = Methane energy output (Mj/ d)

MEI = Metabolizable energy intake

$E_{l(0)}$ = Milk energy output adjusted to zero energy balance(Mj/d)

$E_{l(0)} = E_l + a \times$ energy balance

Where,

E_l = milk energy output

a = 0.95 and -0.84 for positive and negative energy balance, respectively (AFRC, 1990).

The models of Yan et al. (2010) seem to be quite simple and promising for cows consuming grass-based diets. However, as any other empirical model it may in fact restricted to the data set it was developed. Such data set average an intake of concentrates of 498 g/kg DM (range = 198 -869 g concentrate/ kg DM) and have not grazing animals in it; consequently, it applicability to New Zealand pastoral systems may be still limited.

A semi -mechanistic approach.

In an attempt to keep simplicity of estimation and gather the benefit of a more mechanistic approach, Mills et al. (2003) proposed the following model:

$$CH_4 \text{ (MJ/ d)} = a - (a + b) \times e^{-c \times x}$$

Where,

a and b = upper and lower bounds of CH_4 production, respectively.

c = shape parameter determining the rate of change of CH_4 production with increasing ME intake.

Where,

c = - 0.0011 × [starch concentration of the diet/ acid detergent fibre concentration of the diet] + 0.0045

x = Metabolizable energy intake

The application of this non-linear, semi-mechanistic, approach enabled Mills et al. (2003) to represent the typical diminishing response observed as DMI increases, and predicted by the mechanistic models of Danfer et al., (2005), Bannink et al. (2010) and Baldwin's (1995). Such an approach is quite interesting since it combines ease of use and some complexity of mechanistic relationships; therefore increase the applicability of evolving statistical approaches. However, the major barrier for this approach is that most of the statistical models and some of the mechanistic ones require a defined input of intake (Mills, 2008), which in general is not described and practically not measured or poorly estimated under grazing conditions.

Gregorini et al. (2009) used a non-linear approach when attempting to predict herbage intake from grazing dairy cows differing in genetic merit (Breeding worth) at different levels of herbage allowance, during the entire lactation. In this model the level of intake is shaped (diminishing response) by a factor k. This factor represented the hunger drive of grazing dairy cows modulated by genetic merit and stage of lactation. Based on the non-linear approach and suggestions, as well as concerns related the lack of intake inputs; there is a potential linking between the model of Gregorini et al. (2009) with one of the equations (see above) of Yan et al. (2010) or Ellis et al. (2007) to generate a non-linear/ semi-statistical model to easely predict CH_4 production base on pasture intake level (herbage allowance) . Ellis et al. (2007) compiled a large amount of data, comprising 83 beef and 89 dairy datasets from the literature. Although the data was exclusively for northern United States or Canadian research, they developed several simple and multiple linear equations using diet information indicating that the best predictor was the simple linear regression with DMI. Interestingly, their tables show that dietary forage content was one of the best predictors. The study also analysed five other models, including Moe and Tyrell (1979) and Blaxter and Clapperton (1965). Ellis et al. (2007) model performed slightly better than the extant models.

Although not as simple as the model of Mills at al. (2003), or even the proposed link mentioned above, a good example of this semi-mechanistic approach is the recently published work of Volden (2010). NorFor is a semi-mechanistic model of gastro intestinal digestion to optimize cattle nutrient supply (See Volden, 2010 for model equations and details). Volden (2010) demonstrated the usefulness of this semi-mechanistic approach to rapidly assess the effectiveness of different feeding strategies aiming at reducing CH_4, based on rapidly and easy to get data inputs.

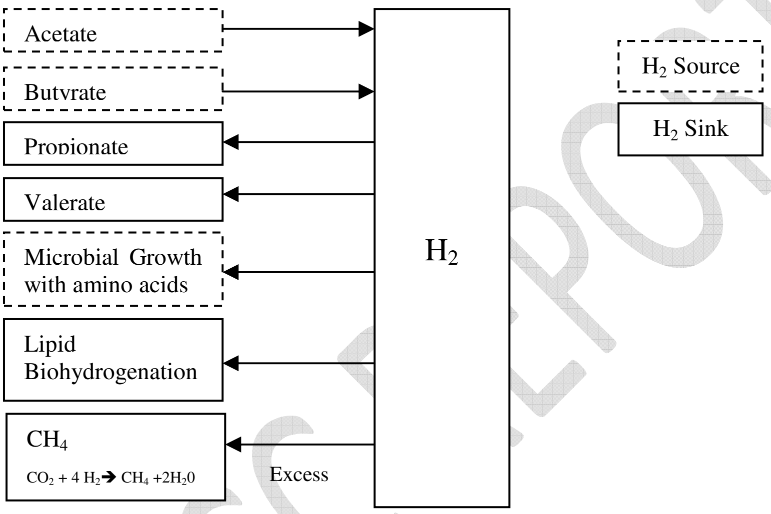
Mechanistic and dynamic models.

Empirical models are set to describe, while mechanistic not only describe the process in question, but also provide (by their nature) understanding (France and Thornley, 1984). Such understanding is mainly given by the nature (*per se*) of mechanistic model to construct relationships (equations) between levels of the organizational hierarchy. For example, as France and Thornley (1984) explain, a mechanistic model describe the behaviour of the level i attributes in term of the attributes of the level i-1. The two levels are connected by a process. The dynamic feature to these types of models is given by simply including the time as variable.

According to Tamminga (2007) dynamic mechanistic models are more suitable and successful for predicting CH_4 emission from ruminants, what is the result of the mechanistic nature of its construction and description of the fermentation process in the rumen (Mills, 2008). There are four major rumen models (standing alone or within a cow model) recognized in the literature: Molly (Baldwin, 1995), Anje/ COWPOL [note: COWPOL is a modification of Anje] (Dijkstra et al., 1992; Benchar et al., 1998; Mills et al., 2001), Karoline (Danfaer et al. 2006) and the model of Martin and Sauvant (2007), with Molly, Anje/ COWPOL and Karoline predicting enteric CH_4 emissions.

The common ancestor. The product of rumen fermentation results in the formation of CH_4 as a sink for excess of hydrogen (Baldwin, 1995). This process of CH_4 formation is described by the following scheme (Figure 1), referred and used by Baldwin (1995), Benchaar et al. (1998) and Mills et al. (2001).

Figure 1: The mechanistic scheme for methane production in the rumen from Baldwin et al., (1987, taken from Mills, 2008).



The CH_4 production is predicted based on the hydrogen balance, which is calculated as follows:

$$Hy_{rumen}\ (mol/d) = Hy_{Hex} + Hy_{AA} - Hy_{MiGr} - Hy_{FA}$$

$$CH_4\ (mol/d) = Hy_{rumen} / 4.0$$

Where,

Hy_{rumen} = hydrogen balance in the rumen

Hy_{Hex} = amount of hydrogen resulting from the fermentation of carbohydrates (hexoses) to VFA.

Hy_{AA} = amount of hydrogen resulting from the fermentation of amino acids in the rumen.

Hy_{MiGr} = amount of hydrogen used for microbial growth

Hy_{FA} = amount of hydrogen used for biohydrogenation of unsaturated fatty acids.

4.0 = mol of hydrogen used to for a mol of CH_4

The main source of hydrogen in this scheme is the amount of hydrogen resulting from the fermentation of nutrients (hexoses and amino acids) to volatile fatty acids (VFA). This amount mostly depends on the nutrients fermentation, stoichiometry coefficients and statistical models describing the rumen conversion of carbohydrates and protein to VFA (Dijkstra et al., 2008). Baldwin et al. (1995) used the stoichiometry models reported by Murphy et al. (1982), when developing Molly. Anje/ COWPOL uses the scheme for methane production from Baldwin et al. (1995), inserted by Benchaar et al. (1998), with a revised (by Bannink et al., 2006) version of the coefficients for the stoichiometry models reported by Murphy et al. (1982). Karoline's coefficients for the stoichiometry models were calculated using in principle the approach of Murphy et al. (1982) for acetate, propionate and butyrate (Dijkstra et al., 2008). And in order to calculate VFA production, the French model of Martin and Sauvant (2007) uses a table of estimations inspired from the data of Murphy et al. (1982) and transformed to equations.

Short description of Karoline, Anje and Molly.

Karoline. The simulation model Karoline, which is a dynamic and mechanistic whole animal model of lactating cows, has been described by Danfer et al. (2006). In brief, Karoline consists of two sub-models, a digestion and a metabolism model. The digestive model of Karoline comprises the forestomachs (rumen) and the intestines (small and large). The metabolism model is represented by the portal drained viscera, liver, mammary gland, muscle, connective and adipose tissue. Karoline is fed with (inputs) crude protein and fat, potentially degradable NDF, starch, fermentation products (silage related) and 'the rest' (other components of OM not accounted for). The crude protein fraction is further detailed by NH_3, amino acids, peptides and soluble and insoluble protein, as well as indigestible protein. Degradation rates of NDF protein and starch need to be provided by the user. The ruminal degradation of carbohydrates and protein and their correspondent passage rates trough the rumen are described by two-compartmental models. The passage rates trough the rumen is regulated by the level of NDF intake and the ruminal degradation of carbohydrates and protein is regulated by the ratio of non-structural and structural carbohydrates. The VFA patterns in Karoline were based on equations derived from a Nordic database (Sveinbjörnsson et al., 2006). These equations are adjusted by level of intake and fat content of supplements fed to Karoline. The methane production in Karoline is calculated on the basis of stoichiometric fermentation equations for both nutrients fermented in the rumen and the hindgut. The predicted methane formation is corrected by the reduction equivalents for microbial cell synthesis, synthesis of microbial fatty acids and hydrogenation of unsaturated dietary fatty acids.

Karoline has been used by Weisbjerg et al. (2005) to predict the CH_4 production from different feed rations. In all cases, the model simulated higher values of CH_4 production than two selected empirical regression equations (IPCC, 1997; Kirchgeßner et al., 1994, taken from Olesen et al., 2005). This observation matches the results of Uden and Danfer (2008), who reported that Karoline, for example, predicts considerably less propionate from sugars, starch and hemicellulose in comparison with Molly. The latest version of Karoline has been changed further from the published version to better represent changes in the stoichiometric fermentation equations for starch and sugar (lower acetate and higher propionate and butyrate formation from starch; lower propionate and higher butyrate formation from sugar). According to Huhtanen (Pekka Huhtanen pers. comm.), Karoline predicts reasonably well changes in CH_4 in response to changes in DMI, proportion of concentrate in the diet, efficiency of microbial protein synthesis and fat supplementation, especially with typical Nordic grass silage-based diets. However, Karoline's CH_4 module still needs further development (Pekka Huhtanen pers. comm.).

Anje. This model is originated from the model of Dijkstra et al. (1992). The later is a dynamic and mechanistic model to simulate the digestion, absorption, and outflows of nutrients in the rumen. Anje does not have the complexity of Karoline (see Danfer et al., 2006) or Molly (see below and Baldwin, 1995). The rumen of Anje (Dijkstra et al., 1992) consists of 17 state variables representing nitrogen, carbohydrate, lipid, and VFA pools. The flux equations are described by Michaelis-Menten or mass action forms. This model includes several specific aspects of rumen metabolism, in particular microbial metabolic activity differentiated by particular populations (amylolityc, fibrolytic and protozoa) and pH-dependent absorption of VFA and ammonia. The model also includes intra-ruminal recycling of microbial matter as a result of protozoa activity (predation) and N recycling via saliva (Bannink et al., 2010). As stated by Benchaar et al. (1998), originally, the model of Dijkstra et al. (1992) did not predict CH_4 emissions. Therefore, Benchaar et al. (1998) incorporated in it the Baldwin's scheme described above. The input parameters to this model are daily DMI, chemical composition of the diet, solubility of protein and starch, degradability and degradation rates of feed components, ruminal passage rates, rumen volume, and rumen pH (Benchaar et al., 1998).

The original improvements (CH_4 production related [Benchaar et al., 1998]) of Anje's rumen were continued by Mills et al., (2001), Bannink et al. (2006; 2010). Mills et al., (2001) added to Anje a module of hindgut CH_4 and revised and incorporated the coefficients for rumen VFA yield described by Bannink et al. (2000). As it was mentioned above, the latter being a revised version of the coefficients generated by

Murphy et al. (1982). Bannink et al. (2008) improved Mills et al., 2001 work by introducing pH-dependent VFA yields from fermentable soluble carbohydrates and starch.

When comparing Anje (Dijkstra et al., 1992) and Molly (Baldwin, 1995); Tamminga et al. (2007) mention two studies, Bannink et al. (1997) and Benchaar et al. (1998) showing better prediction quality for Anje. Tamminga et al. (2007) conclude that Anje, and subsequent adaptations (e.g. Mills et al., 2001), is of a highly mechanistic nature and represents the influence of many key mechanisms in the literature, suggesting Anje as a useful research instrument to study the effectiveness of nutritional measures to reduce CH_4 by cattle.

Recently, Anje was used to investigate the effect of type and quality of grass forage, DMI and proportion of concentrates in dietary DM on variation in CH_4 emission Bannink et al., 2010). Effects of type and quality of fresh and ensiled grass were evaluated by distinguishing two N fertilization rates of grassland and two stages of grass herbage maturity. Simulation results indicated a strong impact of the amount and type of herbage consumed on CH_4 emission, for diets with a proportion of concentrates in dietary DM from 0.1 to 0.4. The lowest emission was established for early cut, high fertilized herbage silage and high fertilized herbage. The highest emission was found for late cut, low-fertilization rate. The N fertilization rate had the largest impact, followed by stage of herbage maturity at harvesting. Simulation results were evaluated against independent data obtained at three different laboratories in indirect calorimetry trials with cows consuming grass herbage mainly. Anje predicted the average of observed values reasonably, but systematic deviations remained between individual laboratories and root mean squared prediction error was a proportion of 0.12 of the observed mean. Anje predicted that emission expressed in g CH_4/ kg DMI decreased upon an increase in dietary N: organic matter ratio. According to Bannink et al. (2010), Anje reproduced reasonably well the variation in measured CH_4 emission in cattle sheds on Dutch dairy farms. Anje's prediction power of CH_4 emissions still needs to be assessed on pastoral systems as the New Zealand one.

Molly. *"... Molly will provide me and associates with a continuum opportunity to learn"* (R. L. Baldwin, 1995). Molly is a mechanistic and dynamic model representing the digestion and metabolism, as well as production of a dairy cow (Baldwin, 1995). The first version of the model (Cow1) was published in 1987 (Baldwin *et al.*, 1987a). Later Cow1 became Myrtle, when the digestion model (Baldwin *et al.*, 1987b) was joined to Cow1. Myrtle could not simulate full lactations. To do so, Myrtle's pool sizes were inflated (see Baldwin 1995) and integration interval was set to 1 day, then becoming Daisy till 1992. Three years later and as a product of Daisy's structural reorganizations, parameter corrections and code reformatting, Molly came to the scene. Since then, Molly has evolved considerably. In brief, the current Molly has evolved to better simulate lipid metabolism (McNamara *et al.*, 2000), lactation curves of New Zealand grazing dairy cows (Palliser *et al.* 2001), photoperiod effect and milk production in grazing dairy cows (Beukes *et al.* 2005), lactation potential (Hanigan *et al.*, 2008), and to properly represent anabolic and catabolic hormone dynamics, and gestational metabolism (Hanigan *et al.*, 2009), as well as the bioenergetics of walking and harvesting herbage while grazing (Gregorini et al., unpublished). Furthermore, the work of Nagorcka et al. incorporated significant elements in Molly (John McNamara pers. com.). Although never fully published, this work expanded bacterial pools, particle dynamics and VFA productions in Molly's rumen (Nagorka *et al.*, 2000) (John McNamara pers. com.).

In the rumen, Molly describes degradation and fermentation of feedstuffs, including cellulose, hemicellulose, starch, soluble sugars, organic acid, and proteins and amino acids. Within the carbohydrate degradation and fermentation processes, production of volatile fatty acids is explicitly described, as well as hydrogen production that is not trapped in VFA. From these equations and the context described in the previous paragraph (and following section), the New Zealand's (DairyNZ) Molly mechanistically predicts enteric CH_4 production.

Previous evaluations of Molly (Baldwin, 1995) under pasture-based diets have indicated under-prediction of enteric CH_4 emissions (Palliser and Woodward, 2002). In the models comparison referred by Tamminga et al. (2007), Benchaar et al. (1998) reported that Molly (Baldwin, 1995) and the rumen model from Dijkstra et al. (1992) were better predictors of enteric CH_4 compared to the empirical models. According to Benchaar et al. (1998) Molly and Dijkstra et al. (1992) had similar R^2 (0.7), however, the prediction error for Molly was higher (37 vs.19.9%), which according the Benchaar et al. (1998) could be eliminated by a correction factor. Both Molly and Anje (Dijkstra et al. (1992) have the same mechanistic module of CH_4 production described above (Figure 1). Therefore, any errors in description of VFA production from any dietary component are compounded in the production of H_2 and thus CH_4. Nevertheless, in fact Molly describes production of CH_4 within the observed ranges for the diets tested and also within 1 to 2 standard deviations of the measurement of CH_4 (John P. McNamara, pers. com).

Recently, Gregorini et al. (2010) tested New Zealand Molly's predictions of enteric CH_4 and urinary N in response to different dietary characteristics determined by grazing and feeding managements (i.e. N fractions, structural and non structural carbohydrates, maize silage feeding, leaf stage of the sward, etc) under New Zealand farming conditions. The results indicate that despite differences between Anje and Molly models; Molly, as a component of a whole farm model (DairyNZ, Beukes et al., 2010), predicts the right and similar trends in enteric CH_4 compared to the model Anje under similar dietary challenges (For an examples, see Dijkstra el al. 2009, Bannink et al. 2010).

Improvements in DairyNZ Molly's CH_4 descriptions will mostly derive from a better description of degradation and fermentation of ingested feedstuffs. In this regard Hanigan et al. (unpublished) used a dataset constructed from the literature to solve for a set of parameters that corrected bias in ruminal pH, ruminal nutrient degradation, microbial growth, and postruminal digestion of Molly. These adjustments had a large impact on model performance as ruminal pH determines the rates of fiber degradation and microbial growth and the latter influences degradation of all nutrients and VFA production. The adjustments also reduced (slightly) the overall prediction error and removed or reduced the slope bias for each of the individual VFA. The balance of VFA dictates the H supply which, in turn, dictates CH_4 production. Thus, such an improvement is promising with respect to improving predictions of CH_4 production and requires further evaluation.

Nagorcka *et al.* (2000) extended the rumen processes in Molly to include three bacterial pools (similarly to Dijkstra et al., 1992), an additional particle size pool, and morphological representation of the herbage consumed by Molly, plus an updated data set on VFA production on grass and pasture-based diets (John P. McNamara, pers. com). This extension resulted in more accurate descriptions of nutrient utilization coming from pasture, however full and systemic analysis of this model has not been possible. Therefore, the latest DairyNZ's Molly (Hanigan et al., unpublished) would benefit from such an approach.

Karoline, Anje and Molly. Advantages and disadvantages

These models offer the potential to describe scenarios and evaluate the intervention strategies for a spectrum of situations, thereby providing a low cost and quick estimate of best practices to mitigate CH_4 emissions. Unfortunately, and despite of the mechanistic feature of these three renown models, the data used in their development (i.e. stoichiometric factors among others) still constraints, the full/ broad context flexibility and applicability expected from a complx and dynamic mechanistic model, at least in regards to enteric CH_4 emissions. Such constraints increase considerably not only according to diet and feeding environment, but also type of cow or even animal (i.e. See Ellis et al. 2009; Kebreab et al., 2008; Levy et al., unpublished). This issue creates imprecision and thereby confounding judgments for each particular model when they are evaluated using the same data set. At the time of validation, however, Molly, Anje and Karoline generally face experimental works with imprecise measurements and results derived from confounding effects, especially under grazing situations. Furthermore, in grazing environments, most of the potentially good data sets come from experiments lacking of a proper experimental design and replication. The later, being generally related to errors/ confusion at the time of determining the experimental unit of the experiment (see Rook, 1999; Lean and Lean, 2010). Consequently, it may not be fair to entirely blame the models when errors of predictions are observed.

What are the advantages and disadvantages of Molly, Anje and Karoline for New Zealand's pastoral systems and the GHG research around it?

Briefly, a good aspect of Molly is its evolution, from the American Holstein TMR fed cow designed by Baldwin et al. in the late 80' to the more flexible (Holstein-Friesian, Jersey and crosses) pasture-fed Molly as described before. Such a metamorphosis lead the pasture fed Molly play a major role in a pastoral whole farm model (WFM, Beukes et al., 2010a) dynamically interacting with climate driven qualitative and quantitative changes in pasture, quality and amounts of bought-in supplements, and its own metabolic capacity to absorb and convert nutrients into milk as determined by age, breed, and genetic merit. This Molly not only behaves according to feed inputs and animal characteristics, but also to common and specific farm management policies and decisions. Recently the WFM was upgraded to include reproductive modeling capability, based on relationships between cow factors, physiology and mating management (Beukes et al. 2010b); and the grazing behaviour of Molly according to sward condition and grazing management (Gregorini et al., unpublished). An issue of Molly still holds on the old stoichiometric coefficient/ models for rumen fermentation pattern (Murphy et al., 1982), the lack of a mechanistic representation of rumen outflows rates, the lack of representation of ionophores and probiotics, the poor representation of the effects polyunsaturated fatty acids, and the lack of representation of other H_2 sinks (i.e. sulfur) with their consequent impact in CH_4 production. The need of the representation of the last four issues in mechanistic models of rumen was raised already by Tamminga et al. (2007) and Ellis et al., (2008). Another issue of Molly is its lack of modularity and thus ‘spaghetti’ features from a software engineering standpoint, which makes the Molly difficult to work with and develop it.

A good aspect of Karoline for New Zealand pastoral systems resides not only in the fact that it is a whole cow, but also in its quite advanced mechanistic approach of its digestive and metabolism modules, as well as the set used to build up the stoichiometric models for rumen fermentation patterns. Such data set is built on the basis of experiments feeding cows with diets mainly composed by grass silage and fresh grass (Sveinbjorsson et al., 2006). Furthermore, as stated by Sveinbjorsson et al. (2006) and

Danfer et al., (2006), Karoline was built on inputs that would not be too difficult to attain in practice, aiming to serve advisory services (Danfer et al., 2006). The latter, in fact, could but not necessary should lead researchers to discard Karoline as a detail research tool. Moreover, despite of showing better behaviour than the stoichiometric models coefficients of Murphy et al. (1982), Karoline's stoichiometric models coefficients still need more refinement, updating and independent evaluation (Dijkstra et al., 2008). As it was mentioned the Karoline's stoichiometric models were built on VFA database is mainly from typical Nordic diets (based on grass silage), while nowadays more whole-crop silages, barley/oats based concentrates with some by-products and protein supplements are fed to cows in the Nordic region (Pekka Huhtanen pers. comm.). With these diets the ranges in fermentation pattern are small, and surprisingly, lactic acid is the most important factor influencing acetate (not starch or proportion of concentrate in the diet). Karoline's old empirical regression equations do 'a better job' in predicting VFA than stoichiometric models used by the newest Karoline (Pekka Huhtanen pers. comm.), which may show the price of keeping Karoline purely mechanistic. An issue of Karoline is that it is built in POWERSIM software. This software is similar to Stella (modelling software), but does not communicate with it, which makes it difficult to connect with other models. POWERSIM communicate Excel spreadsheet, but the recent versions of POWERSIM are made mainly for commercial applications and Karoline does not run in it, at least without some modifications (Pekka Huhtanen pers. comm.).

Anje's rumen is nowadays the most advanced mechanistic rumen model. As it was mentioned above the most interesting and differentiating features of Anje's rumen is the representation of microbial populations (specially protozoa) and the new VFA stoicheometric coefficients from Bannink et al., (2006), as well as the pH dependency of

VFA yield from fermented soluble carbohydrates and starch, incorporated by Bannink et al. (2008). Another good aspect of Anje is also related to the replication of the module of CH_4 production in the hindgut (Mills et al., 2001). Although Anje is been referred as a cow, the metabolic complexity of Molly and Karoline makes Anje look like a rumen model with accessories; what could be a disadvantage. Anje, also lacks of a representation of ionophores and other additives (Tamminga et al., 2007) and still need refinement at the time of assessing the effects of diets with high fat contents (Jan Dijkstra pers. com.). According to Ellis et al. (2009), even the improved coefficient of the stoichiometric models of VFA pattern (Bannink et al. 2006) require adjustment for predicting CH_4 in high grain diets. Moreover, Anje, as Karoline are not incorporated into whole farm models (see below). The most concerning issue of Anje is that requires some manual setting depending on the user knowledge of the simulation context (Andre Bannink, pers. com. SLMACC meeting, Ruakura Research Centre, 2010). Anje's executables or source code is inaccessible when compared with Karoline's and Molly's code accessibility, which presents a disadvantage.

RUMEN THERMODYNAMICS

Does rumen thermodynamics represent a step forward?

Nutrition science comes from medicine, physiology, biochemistry, genetics microbiology, agriculture, home economics and behavioural sciences. Although thermodynamics plays an integral role in the calorimetry of the energy content of the ingested food; paradoxically, thermodynamics is not generally included in such a list (Welch, 1991 at the Symposium on 'History of research in Human energy nutrition'). This absence is also evidenced by the fact that few works in the ruminant nutrition literature (compared to the bulk of literature regarding with rumen function) deals with rumen thermodynamics. Perhaps, it is not the lack of knowledge of the thermodynamics science existence, as demonstrated by several works in energetic of rumen bacteria and rumen redox capacity (See Russell and Cook, 1995, Janssen, 2010), but a simple delay on its incorporation and utilization in modelling of rumen fermentation pattern.

It seems that the awakening of rumen thermodynamics in the modelling side of the story started with the doctoral dissertation of Hoh (1996), as referred by Kohn and Boston (2000). Hoh (1996) integrated equilibrium thermodynamics principles into kinetics models in attempt to explain shifts in the reactions of anaerobic digesters. Due to the relative inconsistency of the VFA stoichiometric model of Murphy et al. (1992), the common ancestor of methane production models (see above) (Kohn and Boston, 2000; Offner and Sauvant, 2006), Kohn and Boston (2000) developed a dynamic model of glucose fermentation to demonstrate the potential for thermodynamic control of rumen fermentation. The reason behind this development resides not only in the inconsistencies the stoichiometric model of Murphy et al. (1992), but also in that a thermodynamic approach would provide 'a fundamental' understanding of the factors altering ruminal fermentation patterns.

Chemical reactions (pathways) are controlled by either thermodynamic or kinetic principles (Chang, 1981, cited by Kohn and Boston, 2000). Thermodynamic principles are based on that, offering the possibility to determine processes direction (pathways) and strength, as well as which process is likely to occur (Welch, 1991, Offner and Sauvant, 2006, Janssen, 2010). Kinetics laws only describe the rate of the reactions (Offner and Sauvant, 2006) and only controls them when they are thermodynamically favourable (Kohn and Boston, 2000) and apply to a microbial monoculture (Offner and Sauvant, 2006). Therefore, in a complex environment/

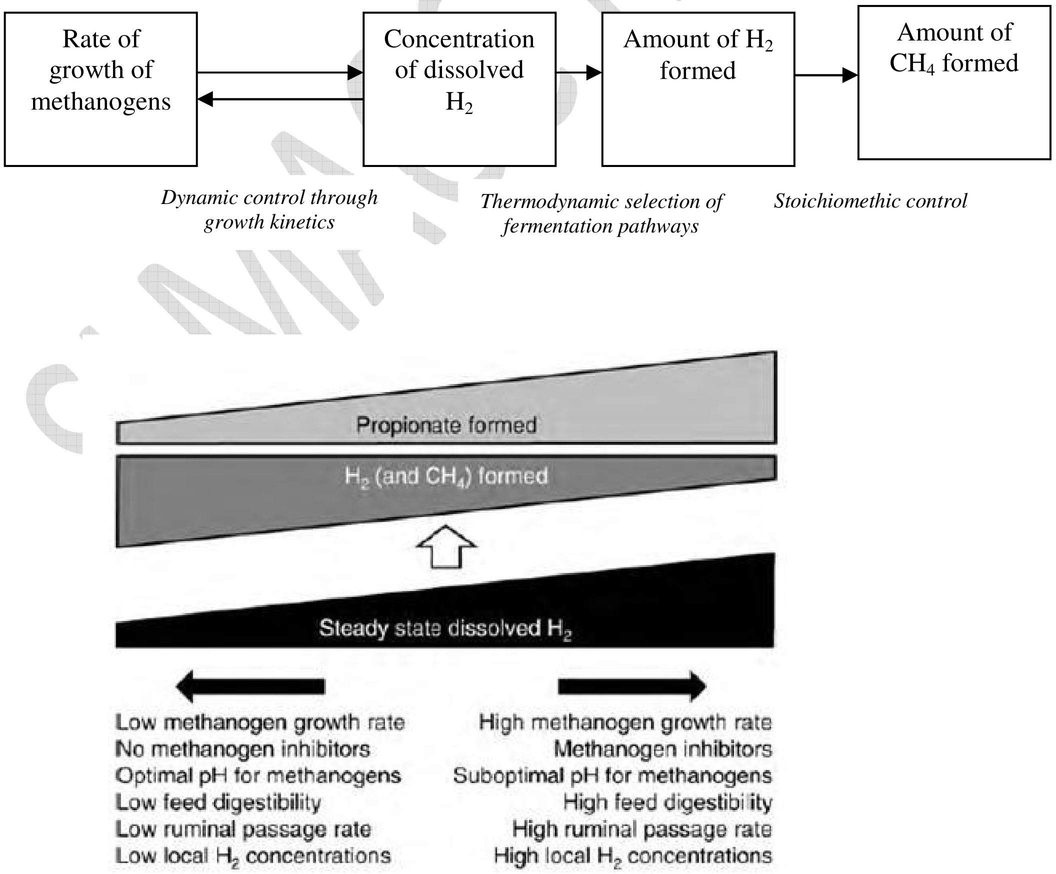
ecosystem like the rumen, with a metabolically diverse potential in the microbiota, thermodynamic laws will probably dictate the success of species, particular metabolic pathways (Jenssen, 2010), and consequently rumen fermentation pattern at any point in time.

Although the glucose fermentation model of Kohn and Boston (2000) predicts realistic concentration of VFA and gasses, the thermodynamic efficiencies of this model were considered static at steady-state (Offner and Sauvant, 2006). Offner and Sauvant (2006) took a step forward from Kohn and Boston (2000) and developed a thermodynamically driven model representing the variation in carbon flows between the VFA, gasses and microbial biomass. This model predicted a satisfactory post-prandial evolution of VFA patterns; however, predictions of pH, and redox potential were less reliable, and predictions of CH_4 were too low.

In a recent thorough literature review, Janssen (2010) evaluated the influence of hydrogen on rumen CH_4 formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. In this work Janssen (2010) proposed a quite integrative and challenging conceptual model (Figure 2), which cries for validation. The model explains the control of CH_4 formation in the rumen by kinetics and thermodynamic laws. Methanogens growth kinetics determines the H_2 concentration, and thermodynamics of the rumen fermentation is controlled by the H_2 concentration. Janseen's conceptual model is exciting and has created huge expectations, especially in the arena of model development.

Neither the model of Kohn and Boston (2000) not the one from Offner and Sauvant (2006) are incorporated into whole rumen (then whole cow) models. And the model of Janssen is still conceptual and warrant mathematical development. Consequently, although the thermodynamic approach of modelling rumen fermentation pattern and then CH_4 is promising, it is still in early stages. Even if they were a component of the rumen/ cow models, they would represent a validation challenge, since there is almost no data (i.e. Gibbs free energy) in this regard, and even less proper experiments set for this purpose. Recently, and based on a thermodynamic principles, Laporte and Gregorini (unpublished) proposed a quite simple approach to asses real-time rumen thermodynamics parameters and then rumen function efficiency using 'easy to get' data (i.e. pH, redox capacity and temperature). If applied, this approach will facilitate data collection not only to evaluate the spatio-temporal efficiency of rumen function, but also validation and model building data.

Figure 2. Janssen's (2010) conceptual model of methanogenesis (Taken from Janssen, 2010).



THE NECESSITY OF SCALING-UP: A FINAL NOTE

Methane predictions through whole farm modelling

Giving attention to scales is important, since simple scaling-up or down leads to errors in phenomena interpretation (Wiens, 1989, Wu, 1999). The integration of CH_4 production from small (rumen) to large (farm) spatio-temporal scales can lead to such a common errors, especially when empirical/ statistical models are used for this purpose, as demonstrated by Ellis et al., (2010). As it was mentioned before; statistical models cannot deal with farm dynamism and complexities. Therefore, and as suggested by Dijkstra et al. (2007) and recently by Ellis et al. (2010), the incorporation of dynamic and mechanistic models of rumen and even better whole cows to ‘whole farm models’ is imperative if we want to progress in the understanding and assessment of CH_4 at a larger scale than the rumen.

Ellis et al. (2010) mentioned the few whole farm models with capabilities to predict CH_4 emissions. For example, FarmGHG (Olsen et al., 2006), DairyWise (Schils et al., 2007a), FarmSim (Saletes et. al., 2004), SIMS Dairy (Schils et al., 2007b) and the WFM (Whole farm model of DairyNZ). From these models, the WFM is the only one that uses a mechanistic and dynamic model of a whole cow, the rest utilize empirical approaches to estimated enteric CH_4 production. The WFM was created more than 10 years ago (Sherlock et al., 1997) using Molly (Baldwin, 1995) as the cow model. The versions of Molly used in the WFM and his current improvements were mentioned before. The WFM has already and is currently being used in New Zealand to set pathways of research, and also by policy makers to re designing pastoral dairy systems for environmental protection (Dave Clark, pers. com.). Readers are referred to Beukes et al., 2010, Gregorini et al., 2010 and Beukes et al. In Press).

FURTHER RESEARCH AND MODEL DEVELOPMENT NEEDED

- Improve stoichiometric component models of rumen fermentation pattern through a new and extensive VFA production data set (not concentrations or molar proportions).
- Categorize stoichiometric component models of rumen fermentation pattern through diet and animal type.
- Develop semi-mechanistic models of methane emission for pastoral systems for quick context/scenario evaluations.
- Describe and mechanistically predict rumen fermentation pattern (i.e. VFA).
- Develop existent rumen fermentation thermodynamic models (Kohn and Boston, 2000 and Offner and Sauvant, 2006) or mathematically build the Janssen's (2010) conceptual model of methanogenesis after thorough concept validation.
- Develop current mechanistic cow models to flexibly represent grazing conditions.
- Incorporate one or a set of mechanistic cow/ rumen models in whole farm models.

RECOMENDATIONS

- Make a functional use of types of models according to the question to answer and the precision and accuracy required.
- The extrapolation of models that are not adequate for describing a specific system to fit experimental observations should be avoided.
- Extensive model parameter calibrations should also be avoided.

Only adequate mechanistic descriptions enable knowledge-based process development and methods to investigate specific questions of interest.

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