

$$dQ_d/dt = I_d(t) - \sum_{d,m} D_d(Q_d, Q_m) - O_d(Q_d)$$

$$dQ_s/dt = I_s(t) + \sum_{d,m} D_d(Q_d, Q_m) + \sum_{m,s} \{ L_m(Q_m, Q_s) - G_m(Q_m, Q_s) \} - O_s(Q_s) - A_s(Q_s)$$

$$dQ_m/dt = \sum_{d,m^*} \{ G_m(Q_m, Q_s) \cdot f_{Gm} + P_m(Q_m, Q_s) \cdot f_{Pm} - L_m(Q_m, Q_s) - P_m(Q_m, Q_s) \} - O_m(Q_m)$$

$$dQ_v/dt = I_v(t) + \sum_{m,m^*,s} \{ G_m(Q_s, Q_m) \cdot [1-f_{Gm}] \cdot f_{Gv} + P_m(Q_m^*, Q_s) \cdot [1-f_{Pm}] \cdot f_{Pv} \} - O_v(Q_v) - A_v(Q_v)$$



Modelling Volatile Fatty Acid Dynamics and Rumen Function in Lactating Cows

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$$\phi = P_{Ac} / (k_{Abs,Ac} + k_{Out,Ac}) + P_{Pr} / (k_{Abs,Pr} + k_{Out,Pr}) + P_{Bu} / (k_{Abs,Bu} + k_{Out,Bu}) + P_{Bc} / (k_{Abs,Bc} + k_{Out,Bc})$$

$$P_{Ac,Vfa} = [P_{Ac} / (k_{Abs,Ac} + k_{Out,Ac})] \times 1 / \phi$$

$$P_{Pr,Vfa} = [P_{Pr} / (k_{Abs,Pr} + k_{Out,Pr})] \times 1 / \phi$$

$$P_{Bu,Vfa} = [P_{Bu} / (k_{Abs,Bu} + k_{Out,Bu})] \times 1 / \phi$$

$$P_{Bc,Vfa} = [P_{Bc} / (k_{Abs,Bc} + k_{Out,Bc})] \times 1 / \phi$$



**Modelling Volatile Fatty Acid Dynamics
and Rumen Function
in Lactating Cows**

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André Bannink

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Abstract

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Mathematical models are developed to quantify and integrate the various processes involved with rumen fermentation. Three extant mechanistic models of rumen fermentation were studied (Baldwin *et al.*, 1987; Danfær, 1990; Dijkstra *et al.*, 1992), each with a truly dynamic representation but different conceptual approach. The models were compared on mathematical representation of individual processes and their prediction accuracy was evaluated. Although the models predicted similar rates of substrate degradation and rumen outflow of organic matter, total crude protein and microbial protein, they differed substantially in representation of the underlying microbial mechanisms. The model of Baldwin *et al.* (1987) performed best in prediction of the combination of rumen pool sizes and duodenal flows, whereas the model of Dijkstra *et al.* (1992) was evaluated to deliver the most realistic outflow of rapidly fermentable carbohydrates. Further, it was identified that all models needed improvement with respect to the prediction of amounts and type of volatile fatty acids (VFA) produced. In a subsequent evaluation it was investigated to what extent individual model elements, of a selection of five, could be responsible for inaccurate VFA predictions. The results suggested that inaccuracy of stoichiometric coefficients of VFA yield from fermented substrate (VFA coefficients) and incorrect representations of VFA absorption kinetics are the most likely causes. New values of VFA coefficients were derived by regression of a stoichiometric model of VFA yield against data of VFA molar proportions observed *in vivo* in rumen fluid of lactating cows. Inputs to the model were observed rates of rumen substrate degradation. Regression against simulated data sets including random error indicated that the accuracy of this method to estimate VFA coefficients is acceptable. Estimates from regressions against *in vivo* data delivered new sets of VFA coefficients for roughage-rich and concentrate-rich diets. In a follow-up study the representation of stoichiometry was made pH-dependent. With regression of this model against *in vivo* data a profound effect of rumen pH on the type of VFA formed from rapidly fermentable carbohydrates was established. Besides VFA production, the rumen concentrations and the amount and profile of VFA available for the cow are also affected by absorption and metabolism of VFA by epithelial tissues in the rumen wall. A mechanistic model was constructed that represents the dynamics of these processes, including the effects of changes in VFA concentration differences between different compartments, the effect of competitive inhibition between VFA and the effect of changes in surface area and epithelial mass. Although some essential characteristics of VFA transport and intra-epithelial metabolism could be reproduced by the model, it was concluded that there is a definite need for more experimental data. Various levels of aggregation need to be included when representing whole rumen function. Besides intrinsic degradation characteristics and passage of ingested substrates, environmental conditions in the rumen and functionality of the rumen wall need to be addressed.

Keywords: Dynamic modelling, Rumen fermentation, Volatile fatty acids, Rumen absorption, Epithelial metabolism, Rumen wall, Lactating cows

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Chapter 1

Introduction to Rumen Function

Introduction to Rumen Function

Feed evaluation systems have been developed to compare the feeding value of feedstuffs and to estimate the amount of feed required by an animal to produce and maintain itself. The methodology in use today has been, and still is, of great benefit for feed manufacturers and farmers because it allows them to optimise ruminant nutrition from a production as well as an economic perspective (Van der Honing & Alderman, 1988). For dairy cows, the feed evaluation systems used in The Netherlands are the system of net energy for lactation (VEM system; Van Es, 1978) and the system for intestinal degradable protein (DVE/OEB system; Tamminga *et al.*, 1994, 2007). Feeding values of forages, compound feeds and by-products are obtained by following prescribed protocols for estimating the feeding value, or by using tabulated values (CVB, 2005a, b).

Inherent in current methodology is the assumption of a unique feeding value for an individual feedstuff or diet dry matter in terms of the amount of energy, protein, or other nutrient that becomes available to the ruminant. Adoption of a unique feeding value implies that important assumptions are made: the feeding value of a feed or feed ingredient is independent of that of others included in the diet, and it is additive which means that the feeding value of the total diet is obtained by weighing feeding values of the dietary ingredients by their relative proportions in the dietary dry matter. However, these assumptions only hold to a limited extent. In general, milk yield is rather sensitive to variation in energy intake when caused by variation in dry matter intake. When the type, i.e. the composition, of diet is changed, however, the effect becomes less predictable. It has been demonstrated that with alteration from starch-rich (maize starch) to fibre-rich (beet pulp) concentrates as supplement to herbage diets (Valk *et al.*, 1990), or with replacement of grass herbage by maize silage (Valk, 1994), the net energy intake observed is not accurately reflected in energy retention in milk and change of body weight. Recently, Yan *et al.* (2003) evaluated current systems of net energy for lactating cows (including the VEM-system) and obtained a mean prediction error of 14% with estimated energy requirements ranging from 30% above or below actual energy intake. Besides, Kebreab *et al.* (2003) analysed energy balance data (652 dairy cow observations) and showed significant differences in estimates of efficiencies of energy conversion compared with previous analyses. Estimates of maintenance requirement of these cows in the 1990s were constantly higher than adopted in current systems. Part of the reason could be genetic differences of cows used in the Kebreab *et al.* (2003) study compared with those in late 1960s and early 1970s. The fact that using the same methodology led to large differences suggests that recommendations made 30 years ago may need to be revised. These findings indicate that the inaccuracy of these net energy systems might be substantial, and that predicted energy requirement and milk yield may differ in the order of 5% to perhaps as much as 10% in certain cases. Animal, environmental or

management factors may have a modifying effect on the extent and the site of feed digestion, on the quantity and type of nutrients absorbed from the gastrointestinal tract and on the utilization of nutrients for maintenance and productive functions. The feeding value of a diet as well as feed requirement for milk synthesis is affected by animal factors such as the level of dry matter intake, the type of diet consumed or the physiological state of the cow. Despite the practical importance of the current VEM and DVE/OEB systems for evaluating feeding value and feed requirements for dairy production, results from feeding trials with rations appropriate to conditions in The Netherlands indicate that relevant variation remains unexplained. Aspects that appear important in the VEM system are type of forage offered (Valk, 1994), characteristics of grass (Bruinenberg, 2003; Kappers & Valk, 1996; Valk *et al.*, 2000) or grass silage (Valk *et al.*, 2006), characteristics of concentrates (Valk *et al.*, 1990; De Visser, 1993) offered, level of glucogenic nutrient supply (Dijkstra *et al.*, 2007a) and distinction between different feeding strategies with a restricted or an *ad libitum* allowance of either roughages and/or concentrates (Rijkema *et al.*, 1990). Also evaluation of the DVE/OEB system indicates that there is room for improvement in the prediction of metabolizable protein and milk protein production. Mean prediction errors appeared to be more than 10% and protein requirements for productive functions were shown to interact strongly with energy metabolism (Van Straalen *et al.*, 1994). Recently, an updated version of the system was introduced (Tamminga *et al.*, 2007). Apart from errors in feed analysis, the apparently unexplainable variation in milk production must originate from either an invalid estimate of energy or nutrient requirements (response to diet) or from an invalid estimate of feeding value of the diet. Almost two decades ago, MacRae *et al.* (1988) reviewed nutrient interactions in dairy cows and concluded that physiological and nutritional factors were not or inappropriately represented in feed evaluation systems and were most likely the causes of the inability of the systems to predict accurately the cow's response to a dietary strategy. In a recent review of the use of soyhulls to replace grain or forages in diets of lactating cows a similar assertion was made in the conclusions (Ipharraguerre & Clark, 2003).

Important factors not represented in current feed evaluation systems are the type of nutrient that is absorbed from the gastrointestinal tract and quantity and type of nutrient available for intermediary metabolism and production functions of the ruminant. The type of nutrient absorbed affects partitioning between maintenance, milk production and body deposition and also the efficiency of energy utilization for productive functions (Dijkstra *et al.*, 2007b; Friggens & Newbold, 2007). Variation in rumen fermentation is a major cause of variation in the quantity and type of nutrients absorbed from the gastrointestinal tract. MacRae *et al.* (1988) ended their review by emphasizing that rumen function in particular deserves attention. Although current systems do take into account variation in the efficiency of microbial protein synthesis (Thomas, 2004; Tamminga *et al.*, 2007), the type of volatile fatty acid (VFA) formed and the effect of rumen conditions on the rumen fermentation process and resulting nutrient profile supplied to the ruminant are still hardly considered. The

importance of variation in rumen function is illustrated when glucose availability for lactating cows needs to be estimated. Dairy cows in early lactation have a high demand for glucose as a precursor of lactose synthesis and the diets offered are generally rich in starch. Feed starch generates glucose by two routes. First, a relatively high proportion of rumen-fermented starch is converted into propionate that, after absorption into portal blood, is almost completely converted into glucose by the liver. Second, the majority of starch bypassing rumen fermentation is digested in the small intestine and subsequently absorbed as glucose. With the first route, up to a quarter of every unit of fermented starch ends up in glucose precursors. With the second route, almost all the starch is converted into glucose but, compared to the first route, a much smaller fraction of this quantity appears in the portal blood because a high proportion of the glucose is metabolized by tissues in the intestinal wall. The difference between both routes of starch digestion indicates the need to estimate glucose delivery to the dairy cow based on an accurate estimate of both the site and extent of starch digestion and the metabolism of glucose precursors by tissues in the gastrointestinal tract (Reynolds *et al.*, 1997; Mills *et al.*, 1999). From reviews on starch digestion (Nocek & Tamminga, 1991) it can be concluded (Reynolds *et al.*, 2001) that there is doubt whether increased post-rumen starch digestion and glucose delivery have a beneficial effect on lactational performance. A serious complication in analysing production trials is that the effect of starch is confounded with other simultaneous nutritional changes. Current methodology that treats rumen digestibility and the fate of rumen digested starch as constants (CVB, 2005a, b; Russell *et al.*, 1992; Van Straalen, 1995) therefore does not suit its purpose because it does not explicitly represent glucose or glucose precursors. Besides, current methodology is not capable of accommodating the detailed nutritional concepts required and does not include the interactions between metabolism of energy, protein and other specific nutrients such as glucose (MacRae *et al.*, 1988). Besides an inadequate prediction of starch digestion and glucose delivery, the amount of metabolizable protein is also susceptible to variation with rumen function. Fermentation conditions and substrate availability in the rumen determine the quantity of microbial protein synthesized as well as the fate of degradable feed protein in the rumen (Dijkstra *et al.*, 1992). The size of both sources of metabolizable protein varies with proteolytic activity of the microbial population, synthesis of microbial matter, and retention time of particulate, fluid and microbial matter. Current methodology takes account of variation in rumen retention time on microbial growth, but for reasons of simplicity this variation is either linked to the level of feed intake (Thomas, 2004) or to specific substrates (Tamminga *et al.*, 2007), but not to both.

From the foregoing, it is clear that the rumen plays a major role in the digestion of feed and that the fermentative processes in the rumen largely determine the site and extent of feed digestion. An appropriate representation of rumen function is essential when predicting response of ruminants to a dietary treatment. The examples presented above indicate that finding explanations for differences among animals and treatments involves examination of

the impact of rumen function on the digestion of feed, the absorption of nutrients from the gastrointestinal tract, and the utilization of nutrients for intermediary metabolism and productive functions.

Rumen function and digestibility

Digestibility of a feed is a crucial factor that needs to be taken into account in every feed evaluation system. Fermentative processes in the rumen are responsible for the main part of the degradation of chemical structures in ingested feed. Reported values of true rumen digestibility indicate that about two third of these structures become degraded and chemically converted. Apparent rumen digestibility is much smaller than true digestibility because substantial amounts of microbial matter synthesized flow out to the small intestine.

Accurate estimation of the impact of rumen function on the extent and site of feed digestion is relevant for three reasons. First, rumen digestion has implications for faecal digestibility and hence the total amount of digestible energy. For example, a depression in rumen degradation of cell wall material may shift its degradation from the rumen to the large intestine and, because of the lower retention time in the hind gut, may reduce whole-tract digestibility of the diet. Such shifts in the site of fermentation have been observed with supplementation of diets with sugars, pectin or starch sources such as maize products and pulp feeds (Valk *et al.*, 1990). Second, between half and two-third of total metabolizable energy (ME) may originate from VFA produced from fermentative degradation by micro-organisms in the rumen. This occurs to a much lesser extent in the large intestine. Type of VFA produced is very much dependent on the type of diet, on the type of substrate fermented, and on the fermentation conditions (Murphy *et al.*, 1984). Third, rumen fermentation strongly dictates the site of digestion of distinct chemical fractions in the feed and hence determines the profile of nutrients absorbed from the gastrointestinal tract, as discussed for starch and protein in the previous paragraph. The fraction that escapes microbial degradation in the rumen depends not only on intrinsic degradation characteristics of these fractions but also on their retention time, rate of comminution and on the activity of the microbial population. As a result, partitioning between the fractions of rumen degraded and the so-called bypass fraction of starch and protein varies with the characteristics of the whole diet.

In conclusion, rumen digestibility not only depends on the types of material ingested and their intrinsic degradation characteristics, but on all the ingredients combined in a diet and on the fermentation conditions encountered in the rumen with that particular diet. Changes in rumen function affect the interactions between individual types of nutrient with respect to their digestibility, site of digestion and availability for the cow's metabolism and productive functions.

Influencing factors and dynamic representation

Variation in rumen function involves dietary as well as animal factors. The distinction between both types of factor is not always clear when defining rumen fermentation conditions. Factors that depend on both the diet and the animal are, for example, outflow rate of fluid and particulate matter, acidity of rumen contents, volume of rumen fluid and absorptive capacity of the rumen wall. With respect to dietary factors, of particular relevance are chemical composition of the diet, intrinsic degradation characteristics of these fractions and structure and type of feed offered (e.g. distinction between roughage and concentrate, particle size). A typical animal factor is level of feed intake, which is related to stage of lactation and genetic potential for milk yield. Besides these factors influencing rumen function, fermentative activity of micro-organisms is important and has been subject to intensive investigation.

Several processes are distinguished when examining rumen function and generally these involve: reduction in size of feed particles, degradation of substrate, utilization of substrate by micro-organisms, synthesis of microbial mass, formation of VFA, ammonia, methane and carbon dioxide as end-products of substrate fermentation, intra-ruminal recycling of microbial matter (death, predation), absorption of VFA and ammonia, and finally, outflow rate of rumen contents. An integrated evaluation of the impact of the factors listed above on rumen function requires that interactions between all influencing factors and rumen processes be represented together. In this respect, lack of integration in empirical or static representations of rumen function remains a serious drawback (MacRae *et al.*, 1988). Therefore, in recent decades several attempts at mechanistic, dynamic modelling have been undertaken in order to achieve such integration (Black *et al.*, 1981; France *et al.*, 1982; Baldwin *et al.*, 1987; Danfær, 1990; Dijkstra *et al.*, 1992; Russell *et al.*, 1992; Lescoat & Sauvant, 1995). Essential to dynamic modelling of rumen function is that the interaction between micro-organisms and substrate utilised is explicitly represented. Because substrate degradation in the rumen involves enzymatically driven processes, concentration of fermentable substrate and that of micro-organisms together determine the rate at which substrate is degraded and utilised by the micro-organisms. Any of the factors mentioned above that affects these concentrations also affects microbial activity. Dynamic models suit this purpose because they define distinct state variables representing size of the microbial population as well as amount of substrate present in the rumen. This allows representation of the concentration dependency of the processes of substrate degradation, microbial growth, and VFA formation and absorption across the rumen wall.

Comparing the dynamic approach of mathematical representation of rumen function with the static approach (either empirical or mechanistic; Russell *et al.*, 1992; Pitt *et al.*, 1996; Van Straalen, 1995) allows qualification of the potential of alternative approaches in explaining variation in rumen function. A first category of models resembles the approach adopted in

current protein evaluation systems and it can be described as empirical and static. These models require the kinetic parameters fractional rate of substrate degradation (k_d) and rumen outflow (k_p) as an input (Ørskov & McDonald, 1979). Despite the use of these input parameters with a kinetic appearance, the model is static in nature and represents no interaction between microbial population and available substrate (Figure 1).

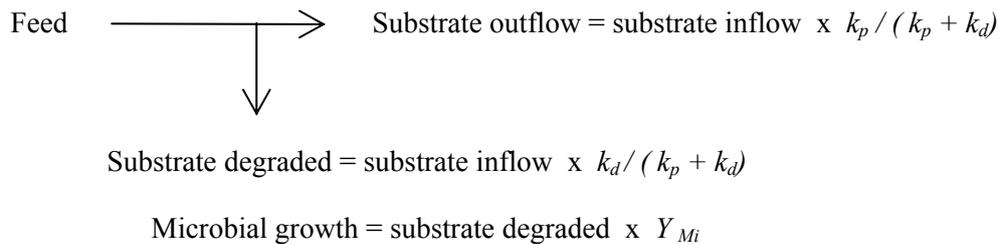


Figure 1. Schematic representation of the method of calculation of partitioning (indicated by arrows) between substrate degradation and outflow, and of microbial growth in static, empirical models of rumen fermentation (adapted from Bannink *et al.*, 2006), with k_p (/d) as fractional rate of passage of substrate, k_d (/d) as fractional rate of degradation of degradable substrate and Y_{Mi} (g microbial matter / kg degraded substrate) as microbial yield from degraded substrate.

A second category of models can be described as (semi-)mechanistic and static. Examples of this type of approach are the CNCPS (Russell *et al.*, 1992; Pitt *et al.*, 1996) and analogous approaches (Van Straalen, 1995; Lescoat & Sauvant, 1995; Danfær *et al.*, 2006). In these models, a more mechanistic representation is used involving equations that include additional factors compared to the first category of models (Figure 2). By differentiating the value of k_d , k_p or Y_{Mi} on the basis of these additional factors, such equations allow for variation in the calculations according to various rumen fermentation conditions. Apart from this ability to differentiate, the input parameters k_d and k_p still fully determine the calculated rate of substrate degradation and outflow and microbial growth however. For the condition of steady-state the fraction of substrate degraded is still calculated by the ratio of $k_d / (k_d + k_p)$ and substrate outflow from the rumen by $k_p / (k_d + k_p)$ (Lescoat *et al.*, 1995; Danfær *et al.*, 2006), or by simple functions of these ratios (Russell *et al.*, 1992; Van Straalen, 1995). Despite more mechanistic representation of these elements than in empirical systems, still no provision is made for mutual interaction between microbial population and substrate availability in the rumen compartment. This category of models therefore lacks a representation of feedback mechanisms between microbial activity and substrate degradation.

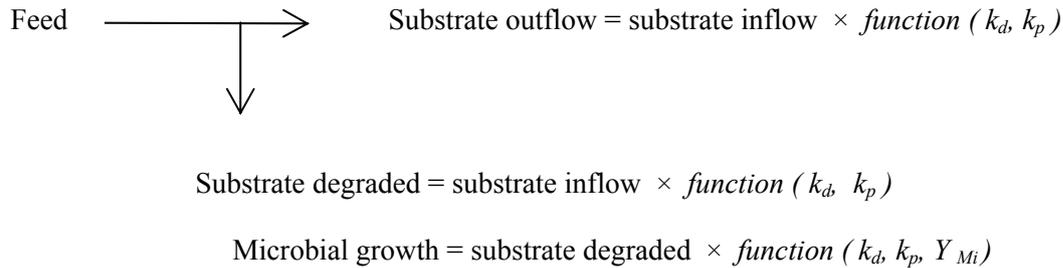


Figure 2. Schematic representation of the method of calculation of partitioning (indicated by arrows) between substrate degradation and outflow, and of microbial growth in static, mechanistic models of rumen fermentation (adapted from Bannink et al., 2006), with k_p (/d) as fractional rate of passage of substrate, k_d (/d) as fractional rate of degradation of degradable substrate and Y_{Mi} (g microbial matter / kg degraded substrate) as microbial yield from degraded substrate.

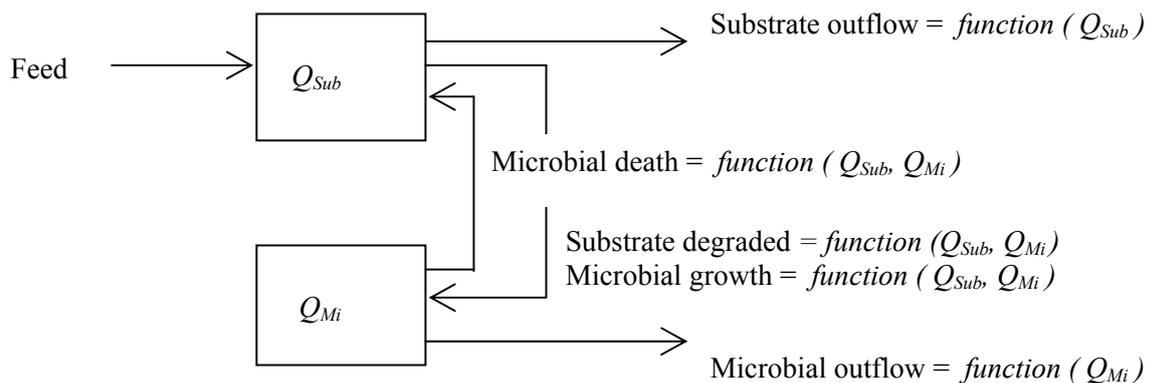


Figure 3. Schematic representation of the method of calculation of partitioning between substrate degradation and outflow, and of microbial growth in dynamic, mechanistic models of rumen fermentation (adapted from Bannink et al., 2006), with boxes Q_{Sub} (kg or mole of degradable substrate) and Q_{Mi} (g of microbial matter) indicating rumen pools and arrows indicating fluxes to, from and between pools.

A third category of models can be described as mechanistic and dynamic (Black *et al.*, 1981; France *et al.*, 1982; Baldwin *et al.*, 1987; Danfær, 1990; Dijkstra *et al.*, 1992) which means that the model calculates rumen pool sizes (concentrations) of microbial matter and available substrate (Figure 3). In this case rates of substrate degradation, microbial growth and rumen outflow are not entirely pre-determined by the value of k_d and k_p but depend on their pool sizes as well. The main focus of these models is to describe the microbial ecosystem in the rumen using a chemostatic representation (France *et al.*, 1982). Enzyme kinetics is applied to describe dependency of substrate degradation and microbial activity on both concentration of substrate and of micro-organisms present in the rumen environment.

Aim of present study and outline of thesis

The aim of the present study was to evaluate and improve the representation of rumen function within dynamic models. The models published by Baldwin *et al.* (1987), Danfær (1990) and Dijkstra *et al.* (1992) were selected as starting points and subjected to further examination. Alternative models published in literature were considered to be either predecessors of these three models, or to represent insufficient detail, or considered static. Although some model comparisons were already available in literature (Kohn *et al.*, 1995; Ramangasoavina & Sauvant, 1993; Dijkstra & France, 1996), these studies lacked detail on scope of evaluation (many trials but few aspects studied, qualitative comparisons only). In Chapter 2, the models are compared on the basis of the type of concepts used and on the impact of non-available input parameters on the simulation results. In Chapter 3, the predictive performance of the models is studied by evaluating them against the same set of independent data. In addition to the model evaluations already published, a theoretical comparison was made of various model characteristics in combination with a fairly complete evaluation on the most relevant aspects of rumen function. The results of this comparison are described in Chapter 4.

Our and other model comparisons indicated that prediction of VFA in particular required further improvement. Therefore, a simulation study was performed to examine the most probable causes of an inaccurate prediction of molar proportions of VFA in rumen fluid (Chapter 5). The simulation results indicated that the most likely cause is inappropriate representation of the stoichiometry of formation of individual VFA with substrate fermentation. In order to derive a stoichiometric representation of rumen fermentation conditions in lactating dairy cows, new parameters were estimated from a database of rumen digestion trials reported in literature with lactating dairy cows only (Chapter 6).

Only part of the VFA produced in the rumen appear in portal blood. In an attempt to obtain a better understanding of the interaction between individual VFA and their rate of metabolism by rumen epithelia, a modelling exercise was performed which represented kinetics of VFA absorption, competitive inhibition among individual VFA in their rate of metabolic activation, and appearance in portal blood. Chapter 7 describes the concepts adopted and the simulations obtained.

The General Discussion in Chapter 8 considers the implications of results described in the preceding chapters for future research and for predicting the response of a cow to dietary treatment. Moreover, the development of dynamic feed evaluation systems based on the representation of individual nutrients is discussed.

References

- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54, 107-131.
- Bannink, A., Dijkstra, J., Kebreab, E. & France, J., 2006. Advantages of a dynamical approach to rumen function to help to resolve environmental issues. In: E. Kebreab, J. Dijkstra, J. France, A. Bannink & W.J.J. Gerrits (Eds.), *Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches*. CAB International, Wallingford, United Kingdom, pp. 281-298.
- Black, J.L., Beever, D.E., Faichney, G.J., Howarth, B.R. & Graham, N.McC., 1981. Simulation of the effects of rumen function on the flow of nutrients from the stomach of sheep: part 1 – description of a computer program. *Agricultural Systems* 6, 195-219.
- Bruinenberg, M., 2003. *Forages from intensively and semi-natural grasslands in the diet of dairy cows*. PhD Thesis Wageningen University, Wageningen, The Netherlands.
- CVB, 2005a. *Handleiding Voederwaardeberekening Ruwvoerders*. Centraal Veevoederbureau, Lelystad, The Netherlands (in Dutch).
- CVB, 2005b. *Veevoedertabel*. Centraal Veevoederbureau, Lelystad, The Netherlands (in Dutch).
- Danfær, A., 1990. *A Dynamic Model of Nutrition Digestion and Metabolism in Lactating Dairy Cows*. PhD Thesis. Beretning fra Statens Husdyrbrugforsøg 671. National Institute of Animal Science, Foulum, Denmark.
- Danfær, A., Huhtanen, P., Udén, P., Sveinbjörnsson, J. & Volden, H., 2006. The Nordic Dairy Cow Model, Karoline – Description. In: E. Kebreab, J. Dijkstra, J. France, A. Bannink & W.J.J. Gerrits (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 383-406.
- De Visser, H., 1993. Characterisation of carbohydrate in compound feeds. In: P.C. Garnsworthy & D.J.A. Cole (Eds.), *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham, United Kingdom, pp. 19-38.
- Dijkstra, J., Neal, H.D.StC., Beever, D.E. & France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *Journal of Nutrition* 122, 2239-2256.
- Dijkstra, J. & France, J., 1996. A comparative evaluation of models of whole rumen function. *Annales de Zootechnie*, 45, Suppl. 1, 175-192.
- Dijkstra, J., Kebreab, E., Bannink, A., Crompton, L.A., López, S., Abrahamse, P.A., Chilubrost, P., Mills, J.A.N., & France, J., 2007a. Comparison of energy evaluation systems and a mechanistic model for milk production by dairy cattle offered fresh grass-based diets. *Animal Feed Science and Technology*, in press (doi:10.1016/j.anifeedsci.2007.05.011).

- Dijkstra, J., Kebreab, E., Mills, J.A.N., Pellikaan, W.F., López, S., Bannink, A. & France, J., 2007b. Predicting the profile of nutrients available for absorption: from nutrient requirement to animal response and environmental impact. *Animal* 1, 99-111.
- France, J., Thornley, J.H.M. & Beever, D.E., 1982. A mathematical model of the rumen. *Journal of Agricultural Science, Cambridge* 99, 343-353.
- Friggens, N.C. & Newbold, J.R., 2007. Towards a biological basis for predicting nutrient partitioning: the dairy cow as an example. *Animal* 1, 87-97.
- Ipharraguerre, I.R. & Clark, J.H., 2003. Soyhulls as an alternative feed for lactating dairy cows: a review. *Journal of Dairy Science* 86, 1052-1073.
- Kappers, I.E. & Valk, H., 1996. The effect of N-fertilization on feeding value, feed intake and N-utilization of grass in dairy cows. 2. Results of indoor feeding trials. Report ID-DLO 274, Institute for Animal Science & Health, Lelystad, The Netherlands.
- Kebreab, E., France, J., Agnew, R.E., Yan, T., Dhanoa, M.S., Dijkstra, J., Beever, D.E. & Reynolds, C.K., 2003. Alternatives to linear analysis of energy balance data from lactating dairy cows. *Journal of Dairy Science* 86, 2904-2913.
- Kohn, R.A., Boston, R.C., Ferguson, J.D. & Chalupa, W., 1995. The integration and comparison of dairy cow models. In: A. Danfaer & P. Lescoat (Eds.), *Proceedings IVth International Workshop on Modelling Nutrient Utilization in Farm Animals*, National Institute for Animal Science, Foulum, Denmark, pp 117-128.
- Lescoat, P. & Sauvant, D., 1995. Development of a mechanistic model for rumen digestion validated using duodenal flux of amino acids. *Reproduction Nutrition and Development* 35, 45-70.
- MacRae, J.C., Buttery, P.J. & Beever, D.E., 1988. Nutrient interactions in the dairy cow. In: P.C. Garnsworthy (Ed.), *Nutrition and Lactation in the Dairy Cow*. Butterworths, London, United Kingdom, pp. 55-75.
- Mills, J.A.N., France, J. & Dijkstra, J., 1999. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 1. Dietary starch characterisation and ruminal starch digestion. *Journal of Animal and Feed Sciences* 8, 291-340.
- Murphy, M.R., 1984. Modeling production of volatile fatty acids in ruminants. In: R.L. Baldwin & A.C. Bywater (Eds.), *Modeling Ruminant Digestion and metabolism in Ruminants*. University of California, Davis, United States of America, pp. 59-62.
- Nocek, J.E. & Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *Journal of Dairy Science* 74, 945-958.
- Pitt, R.E., Van Kessel, J.S., Fox, D.G., Pell, A.N., Barry, M.C. & Van Soest, P.J., 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. *Journal of Animal Science* 74, 226-244.

- Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science, Cambridge* 92, 499–503.
- Ramangasoavina, B. & Sauvant, D., 1993. Comparative validation of 3 models of rumen digestion to predict the duodenal microbial N flows. *Annales de Zootechnie* 42, 164-165.
- Reynolds, C.K., Sutton, J.D. & Beever, D.E., 1997. Effects of feeding starch to dairy cattle on nutrient availability and production. In: P.C. Garnsworthy & J. Wiseman (Eds.), *Recent Advances in Animal Nutrition, Proceedings 30th University of Nottingham Conference for Feed Manufacturers*. University of Nottingham Press, Nottingham, United Kingdom, pp. 105-134.
- Reynolds, C.K., Cammell, S.B., Humphries, D.J., Beever, D.E., Sutton, J.D. & Newbold, J.R., 2001. Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. *Journal of Dairy Science* 84, 2250-2259.
- Rijkema, Y.S., Van Reeuwijk, L. & Goedhardt, P.W., 1990. Effects of pattern of concentrate feeding on milk production. *Netherlands Journal of Agricultural Science* 38, 461-474.
- Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J. & Sniffen, C.J., 1992. A Net Carbohydrate and Protein System for evaluating cattle diets. 1. Ruminant fermentation. *Journal of Animal Science* 70, 3551-3561.
- Tamminga, S., Van Straalen, W.M., Subnel, A.P.J., Meijer, R.G.M., Steg, A., Wever, C.J.G. & Blok, M.C., 1994. The Dutch protein evaluation system: the DVB/OEB-system. *Livestock Production Science* 40, 139-155.
- Tamminga, S., Brandsma, G.G., Dijkstra, J., Van Duinkerken, G., Van Vuuren, A.M. & Blok, M.C., 2007. Protein evaluation for ruminants: the DVE/OEB 2007 system. CVB Documentation report 53. Centraal Veevoeder Bureau, Lelystad, The Netherlands, pp. 65.
- Thomas, C., 2004. *Feed into Milk: A new applied feeding system for dairy cows*. Nottingham University Press, United Kingdom, p. 72.
- Valk, H., Klein Poelhuis, H.W. & Wentink, H.J., 1990. Fibrous and starchy carbohydrates in a diet for dairy cows. *Netherlands Journal of Agricultural Science* 38, 475-486.
- Valk, H., 1994. Effects of partial replacement of herbage by maize silage on N-utilization and milk production of dairy cows. *Livestock Production Science* 40, 241-250.
- Valk, H., Leusink-Kappers, I.E. & Van Vuuren, A.M., 2000. Effect of reducing nitrogen fertilizer on grassland on grass intake, digestibility and milk production of dairy cows. *Livestock Production Science* 63, 27-38.
- Valk, H., Klop, A., Hindle, V.A. & Mathijssen-Kamman, A.A., 2006. Invloed van voeropnameniveau op de pensfermentatie en vertering van hoogverteerbare graskuilen aangevuld met mengvoeders bestaande uit langzaam- of snelfermenteerbare grondstoffen. ASG Intern rapport 04. Animal Sciences Group, Wageningen UR, Lelystad, The Netherlands, p. 30 (*in Dutch*).

- Van der Honing, Y. & Alderman, G., 1988. Systems for energy evaluation of feeds and energy requirements for ruminants. *Livestock Production Science* 19, 217-278.
- Van Es, A.J.H., 1978. Feed evaluation for ruminants. I. The system in use from May 1977 onwards in The Netherlands. *Livestock Production Science* 5, 331-345.
- Van Straalen, W.M., Salaün, C., Veen, W.A.G., Rijpkema, Y.S., Hof, G. & Boxem, T.J., 1994. Validation of protein evaluation systems by means of milk production experiments with dairy cows. *Netherlands Journal of Agricultural Science* 42, 89-104.
- Van Straalen, W.M., 1995. *Modelling of Nitrogen Flow and Excretion in Dairy Cows*. PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- Yan, T., Agnew, R.E., Murphy, J.J., Ferris, C.P. & Gordon, F.J., 2003. Evaluation of different energy feeding systems with production data from lactating cows offered grass silage – based diets. *Journal of Dairy Science* 86, 1415-1428.

Chapter 2

Impact of Diet-Specific Input Parameters on Simulated Rumen Function

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Impact of Diet-Specific Input Parameters on Simulated Rumen Function

Abstract

Theories and concepts were investigated that have been applied in three extant models of rumen function by Baldwin et al., Danfær and Dijkstra et al., as a preliminary step to investigate theories of intake regulation. These models are the most detailed at present and differed particularly in the description of microbial metabolism. Simulations were performed on inputs derived from seven experimental diets with very complete observations available of rumen dynamics. Comparison between models indicated that their simulation results differed markedly. In addition to daily feed intake and feed composition as model inputs, each model requires its own set of parameter inputs. However, some parameter inputs could not be estimated accurately from the available observations. The role of these unknown inputs on simulation results was studied by manipulating their estimated value. It was concluded that, in particular, parameter inputs whose concepts do not correspond to rumen observations have a large impact on model behaviour. Therefore, models need to be developed further to versions that use parameter inputs that can be readily estimated from rumen observations. The goal of the investigated rumen models is to simulate the chemical transactions inside and the nutrient release from the rumen. Nutrients absorbed after digestion seem to be important factors for regulation of daily intake. However, the models take daily feed intake as a known input instead of describing the regulation of intake, although it is highly unpredictable in practice. Thus, extant rumen models need to be developed and evaluated further before they can be used to investigate theories that go beyond the rumen compartment.

Introduction

Current developments in ruminant production systems indicate an increasing need for a more efficient production with a minimum of environmental pollution. To achieve this in practice and to be able to predict the consequences of proposed alterations in the management of production systems, more knowledge is required about the control of feed intake and the metabolic fate of nutrients absorbed from the gastrointestinal tract after feed digestion. Feed evaluation systems that are used in current practice of ruminant production systems do not yet consider the profile of nutrients absorbed or their temporal pattern of absorption, or a variety of factors that have been identified to control feed intake.

Mechanisms responsible for the control of feed intake have been studied extensively and theories have been formulated (Gill & Romney, 1994) for control of intake in the short term (meal sizes and pattern of meals) and the long term (average daily intake). Poppi *et al.*

(1994) integrated theories on physical and metabolic factors thought to be involved in the limitation of feed intake and for which mathematical relationships could be derived. Their model requires the profile of absorbed nutrients after feed digestion as an input.

With ruminants, the rumen compartment of the gastrointestinal tract in particular is important for understanding feed digestion and the profile of absorbed nutrients. Because of extensive microbial activity in the rumen, feedstuffs that would be indigestible by the digestive enzymes secreted by the animal are digested by microbial enzymes. In this way as much as 70-80% (Czerkawski, 1986) of largely indigestible feed is converted into microbial organic material (OM; dry matter minus ash) and into volatile fatty acids (VFA) as end-products of microbial fermentation. The absorbed VFA can account for up to two thirds of the ruminant energy requirement (Sutton, 1985) and the absorbed microbial protein can account for up to 90% of ruminant protein requirements (Czerkawski, 1986).

Consequently, rumen modelling is an essential part of investigating theories about feed digestion, nutrient absorption and intake regulation. Considerable progress has already been made with the most sophisticated extant rumen models by Baldwin (1995), Danfær (1990) and Dijkstra *et al.* (1992). However, before using them to investigate current theories that go beyond the rumen compartment, several issues still need to be addressed. First, the mechanism of microbial fermentation, with the conversion of feed matter into microbial matter and large amounts of VFA, is critical for a correct description of rumen function. However, until now, VFA prediction has been inaccurate (Dijkstra, 1994a) and thus the models must be improved in this aspect. Second, the profile of nutrient release predicted by the rumen models cannot be considered as the profile appearing in the bloodstream because part of the absorbed VFA is metabolized in the rumen wall (Bergman, 1990). Third, the models are not fully self-explanatory in that crucial rumen processes are described with diet-specific parameters that are required as model input, in addition to input variables of feed intake and feed composition. However, a number of these parameter inputs are difficult to estimate from experimental data. This paper addresses the last issue. The impact of these parameters on model behaviour was investigated together with the theories and concepts applied. This is a preliminary step in the further development of these models and their use in the investigation of theories of intake regulation.

Extant Rumen Models

Model inputs and simulations

Of the three models considered, the models of Baldwin *et al.* (1987; BA) and Danfær (1990; DA) are more comprehensive than that of Dijkstra *et al.* (1992; DY), in that they do not only integrate theories on rumen function but also describe post-rumen digestion processes, intermediary metabolism and ruminant production (milk, growth, reproduction). In

this study the rumen part was isolated with the initial values for urea concentration in blood and saliva. The basic organizational structure of these rumen models is depicted in Figure 1. The BA and DY models were re-coded using the simulation language CSMP (Speckhart & Green, 1976) and run on a VAX computer. Simulations to steady-state were performed with a fourth-order variable-step-length Runge-Kutta as integration method, integration periods for the BA, DA and DY model of 20, 50 and 20 days, respectively, and constant inputs. Required model inputs were derived from detailed observations of daily means of rumen contents and flows in lactating Dutch Friesian dairy cows on seven grass based diets at average intake levels and average to high nitrogen contents (Table 1). Diets 1 to 3 (Van Vuuren *et al.*, 1993) involved the partial substitution of fresh-cut grass (diet 1) by maize meal (diet 2) or beet pulp (diet 3). Fresh grass diets 4 to 7 (Van Vuuren *et al.*, 1992) differed in harvesting period (diets 4 and 5 in Summer, diets 6 and 7 in Autumn) and N fertilization rate (diets 4 and 7 high and diets 5 and 6 low N fertilization).

In addition to the input variables of feed intake and feed composition, each model requires a specific selection of the following parameter inputs that can readily be estimated from the observations. Rumen liquid volume, potentially degradable and soluble fraction of neutral detergent fibre (cell wall carbohydrates; NDF), starch (ST), soluble carbohydrates (SC) and protein (P), fractional passage rates of rumen liquid and solids, and rumen pH and the minimum pH value reached during the day. However, in addition to these inputs every model requires parameter inputs that are not available from ruminal observations (BA and DA models) or remain uncertain (DY model).

Table 1. Input variables for extant rumen models.

	Diet	1	2	3	4	5	6	7
DMI		16.2	16.3	16.5	13.3	16.8	13.0	15.2
	Ryegrass	14.5	9.3	9.3	12.4	15.9	12.1	14.3
	Maize	0	5.3	0	0	0	0	0
	Beet pulp	0	0	5.5	0	0	0	0
	Concentrate	1.7	1.7	1.7	0.9	0.9	0.9	0.9
Composition								
	SC	146	104	190	164	174	146	193
	ST	8	157	23	5	4	5	4
	NDF	409	351	407	373	397	406	333
	N	29	27	26	34	28	27	31

Input variables for extant rumen models with daily intake (kg of dry matter /d, DMI) and soluble carbohydrates (SC), starch (ST), neutral detergent fibre (NDF) and N content (g /kg of DMI) of diet 1 through 7.

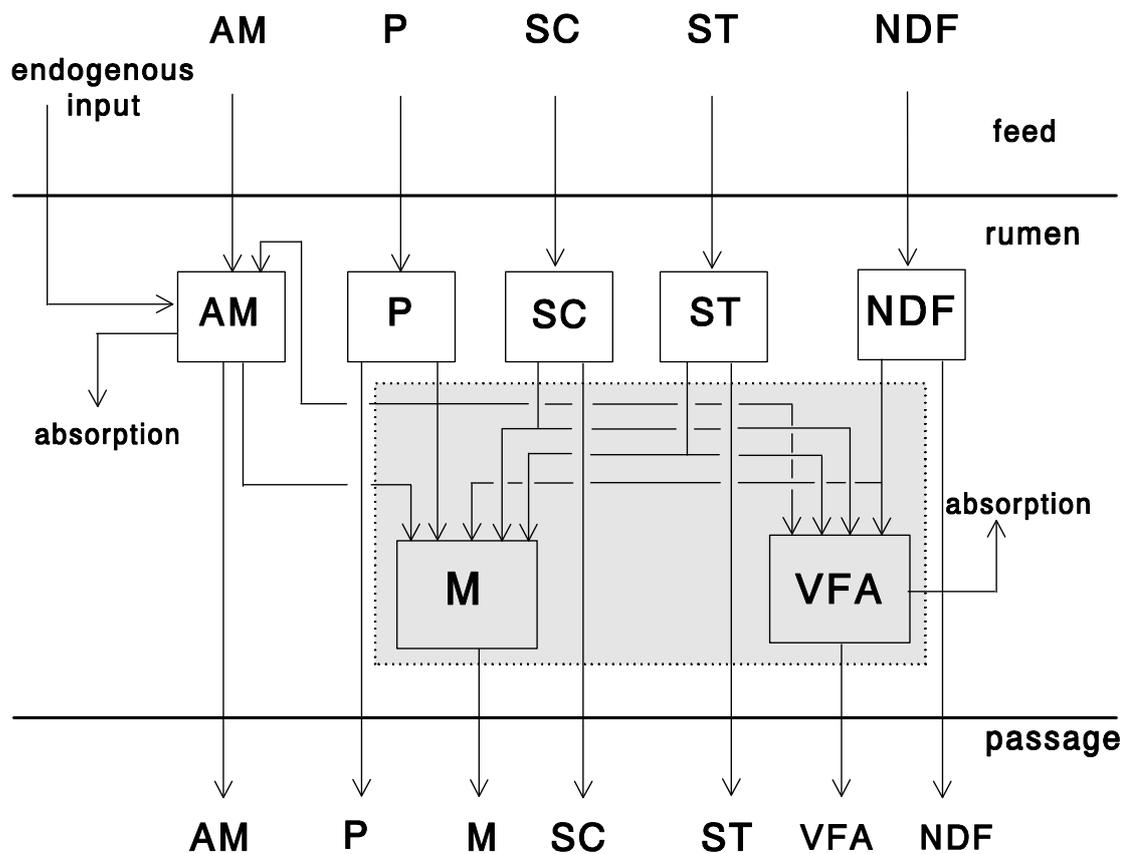


Figure 1. Basic scheme of extant rumen models. Main distinctions between the BA, DA and DY models occur in the dotted square, which is represented in detail in Figure 2. AM, ammonia; P, protein; SC, soluble carbohydrates; ST, starch; NDF, neutral detergent fibre; M, microbial mass; VFA, volatile fatty acids.

Uncertain parameter inputs in BA Model

The BA model contains a description of particle dynamics in addition to the chemical transactions in the rumen (Figure 2A), parameterized with $k_{lp,sp}$ (/d) for the fractional comminution rate of the quantity of large particles in the rumen (Q_{lp} , g) to that of small particles (Q_{sp} , g; usually defined as passing a sieve with 1-2 mm pore size) and f_{sp} for the fraction of small particles in ingested food. In the model, it is assumed that only small particles flow to the duodenum, whereas the observed fractional passage rate of rumen solids ($k_{p,sol}$, /d, in BA1; see Table 2; Van Vuuren *et al.*, 1992, 1993) relates not merely to small particles but to the total content of rumen solids, and in this respect model concepts are not compatible with observations. To rectify this, with steady-state simulation, $k_{p,sol}$ can be multiplied by the ratio of the total particle pool size to the small particle pool size,

$k_{kp.sol} \times (Q_{lp} + Q_{sp})/Q_{sp}$, but only when Q_{lp} and Q_{sp} are simulated reasonably (BA2; Table 2). Thus, the model parameter of small particle passage can be calculated using observed values. The model was evaluated with and without this amendment of the model parameter of small particle passage (BA1 and BA2, respectively; Table 2) using Baldwin's original parameter values of 4.5 /d and 0.4 for $k_{lp,sp}$ and f_{sp} , respectively. However, these values could have become unrealistic due to this amendment, because the assumption of reasonable Q_{lp} and Q_{sp} might not hold. Since more appropriate parameter values cannot be derived from observations, a sensitivity analysis has been performed on $k_{lp,sp}$ and f_{sp} with inputs of diet 1 (values chosen as optimal were used in BA3; Table 2).

Uncertain parameter inputs in DA model

Instead of using observed fractional passage rates of rumen fluid ($k_{p,liq}$, /d) and solids ($k_{p,sol}$, /d), Danfær (1990) estimated distinct fractional passage rates for the identified rumen pools that are all independent from each other (DA1; Table 2). In order to let the DA model follow observed rumen conditions like the BA and DY models, parameters were substituted with observed values (DA2, DA3 and DA4; Table 2). However, some adaptations had to be made for this, concerning calculation of passage rate of pools with combined soluble and insoluble, degradable P, with combined soluble and insoluble, degradable ST, and with microbial matter. Danfær's original estimates of the fractional passage parameter, $k_{p,s}$ (/d), of the quantity of substrate s in the rumen (Q_s , mol of N or C) including soluble as well as insoluble, degradable substrate, is reformulated from $Q_s \times k_{p,s}$ to $Q_s [k_{p,liq} \times f_{liq} + k_{p,sol} (1 - f_{liq})]$, where f_{liq} is the soluble fraction of substrate s in the diet. A fractional microbial passage rate of $k_{p,liq}/2$ gave the most realistic results from several alternatives ($k_{p,liq}$, $k_{p,sol}$, $k_{p,sol} \times 2$, $k_{p,liq}/2$ and, $(k_{p,sol} + k_{p,liq})/2$) and was closest to Danfær's original values. In general rumen models are very sensitive to changes in passage rates (Baldwin *et al.*, 1987; Dijkstra *et al.*, 1992) and, although not completely equivalent to the other two models, adaptations to the model in this study provided parameterization of microbial and substrate passage rates compatible with observed values. Another important concept in the DA model is the set of equations describing the partitioning of microbial fermentation rate of SC, ST and NDF into VFA (Figure 2B) with (1) mass of rumen microbial protein (Q_{mp} , mol of N), and rumen SC, ST and NDF available for microbial utilization (Q_{sc} , Q_{st} and Q_{ndf} , respectively, mol of C) as state variables, and (2) Michaelis-Menten parameters a (g/d) and b (g) in Equation [1] for total rate of carbohydrate fermentation into VFA (R_{tf}) and parameter c (no dimension, between 0 and 1) in Equations [2], [3] & [4] for the partitioning of R_{tf} into fermentation rate of SC, ST and NDF:

$$\text{Total rate of carbohydrate fermentation into VFA (mol C /d) = } \\ R_{tf} = a \times Q_{mp} \times [(Q_{sc} + Q_{st} + Q_{ndf}) / (b + (Q_{sc} + Q_{st} + Q_{ndf}))] \quad [1]$$

$$\text{Fermented SC (mol C /d)} = R_{tf} \times Q_{sc}/(Q_{sc} + Q_{st}) \times e^{[-c \times Q_{ndf}/(Q_{sc} + Q_{st})]} \quad [2]$$

$$\text{Fermented ST (mol C /d)} = R_{tf} \times Q_{st}/(Q_{sc} + Q_{st}) \times e^{[-c \times Q_{ndf}/(Q_{sc} + Q_{st})]} \quad [3]$$

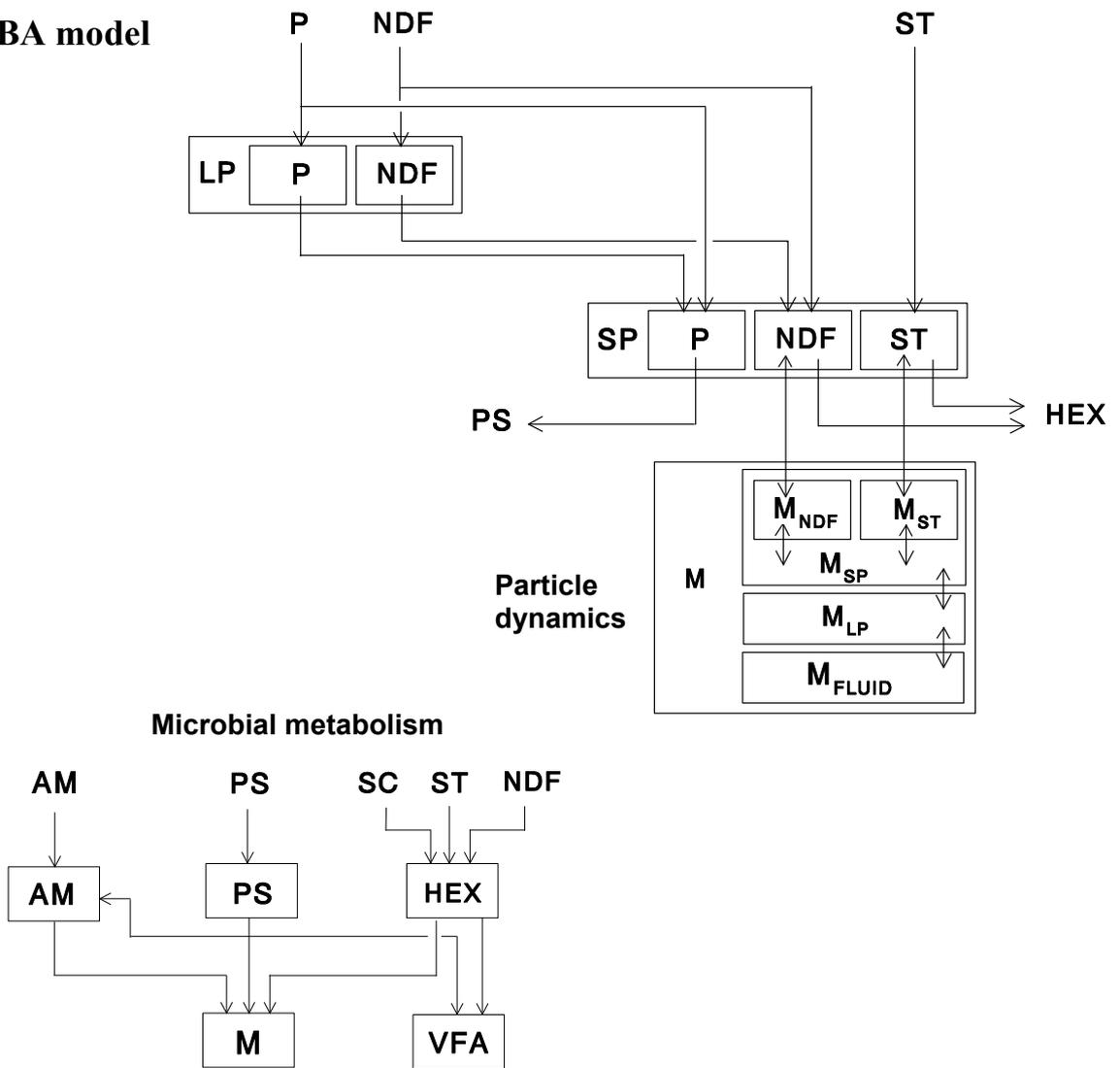
$$\text{Fermented NDF (mol C /d)} = R_{tf} \times (1 - e^{[-c \times Q_{ndf}/(Q_{sc} + Q_{st})]}) \quad [4]$$

These empirical equations provide for a decreased proportion of NDF fermentation with an increased proportion of easily fermentable SC and ST in the diet, and vice versa. The value of the parameter c is dependent on the diet, but no indication is given as to how to obtain a proper value. A preliminary estimate of 0.04 was calibrated by Danfær (1990) to a small value of 0.0006. To investigate the precise impact of changing c values, a simulation with a relatively high c value of 0.4 was added to simulations with the c values used by Danfær. Simulation results were compared for these three c values comprising a wide range (DA2, DA3 and DA4, respectively; see Table 2).

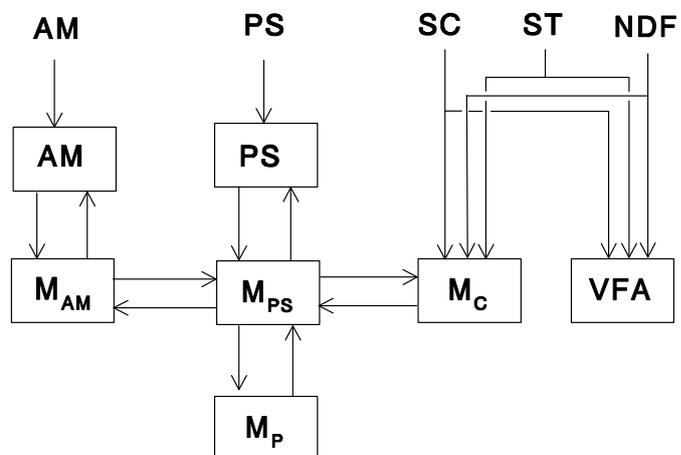
Uncertain parameter inputs in DY model

The DY model requires parameter inputs that are routinely measured in current research on rumen digestion. However, differences in experimental methods can have a strong influence on the observed values. The sampling schedule of rumen pH determines the accuracy of extrapolation of observed values to obtain estimates of the required pH parameters. The rumen pH and its standard deviation are frequently reported, and using these observations the daily mean pH and minimum pH during the day can be estimated. However, in the case of strong fluctuations of rumen pH, or in the case where only a small number of sampling points of rumen pH are available, as well as the situation where daily pH patterns are not even published, the time interval in which pH is below 6.3 ($T_{6.3}$, h) and the interval below 6.0 ($T_{6.0}$, h) are prone to estimation error. A smaller $T_{6.0}$ value increases the fraction of protozoa in the amylolytic microbial pool (MA) containing both amylolytic bacteria and protozoa (BA and PO, respectively) that grow on SC and ST (Figure 2C), and also increases protozoal death and predation on cellulolytic bacteria (BC) growing on NDF (Figure 2C) and consequently intra-ruminal recycling. A larger $T_{6.3}$ value decreases the rate of NDF degradation, because cellulolytic activity is reduced below pH 6.3. Therefore, in addition to what seem to be the best estimates of $T_{6.3}$ and $T_{6.0}$ (DY1, Table 2), use of the extreme combination of the largest possible $T_{6.3}$ and the smallest possible $T_{6.3}$ within the limits of the few observed values, gives the largest possible shift in simulated model outcome (DY2, Table 2).

A; BA model



B; DA model



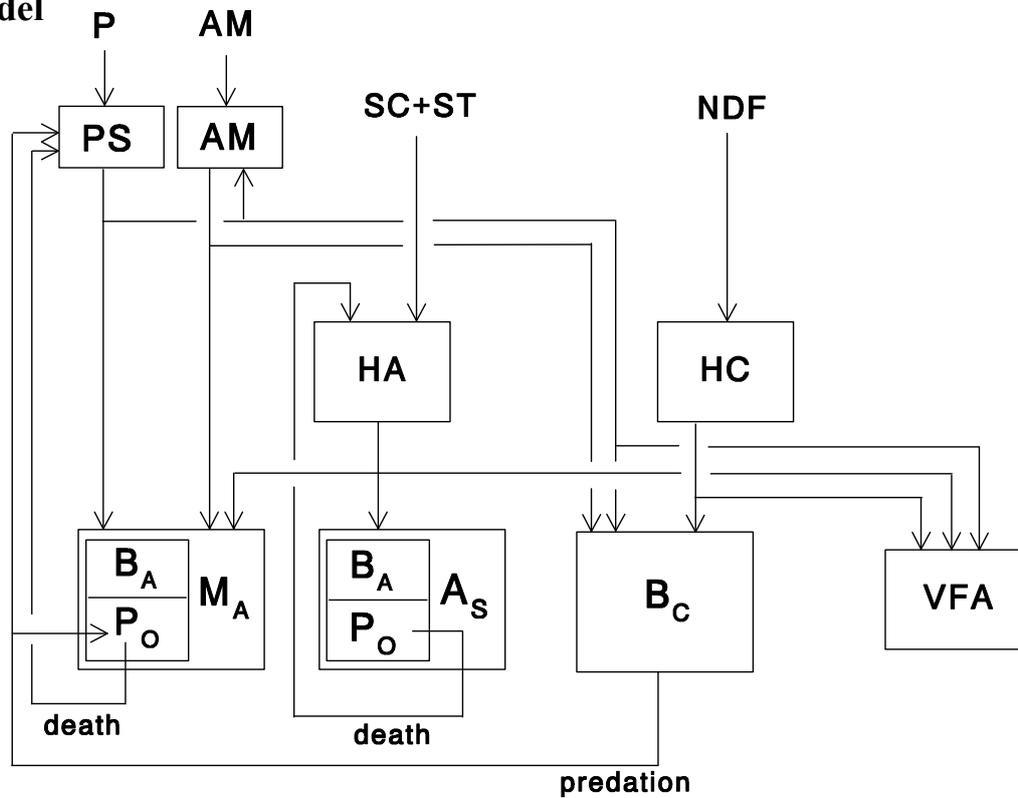
C; DY model

Figure 2. (A) Detailed representation of the microbial mechanism of the dotted square shown in Figure 1 in the BA model. AM, ammonia; HEX, soluble rumen hexose; M, microbes; MLP, microbes bound to LP; MNDF, microbes bound to NDF; MSP, microbes bound to SP; MST, microbes bound to ST; MFLUID, microbes suspended in fluid; NDF, neutral detergent fibre, LP, large particles; P, protein; PS, soluble protein; SC, soluble feed carbohydrates; SP, small particles; ST, starch; VFA, volatile fatty acids. Passage and absorption flows (see Figure 1) not represented. Double-headed arrows indicate a two-way interaction between particulate and microbial matter (MST and MNDF) or equilibrium equations for the partitioning of microbial matter over the physical compartments (MLP, MSP and MFLUID). (B) Detailed representation of the microbial mechanism of the dotted square in Figure 1 in the DA model. MAM, microbial ammonia, MC, microbial lipid, starch and cell wall; MPS, microbial free protein; MP, microbial macromolecular protein. Other abbreviations as described for Figure 2A. Passage and absorption flows (see Figure 1) are not represented. (C) Detailed representation of the microbial mechanism of the dotted square shown in Figure 1 in the DY model. AS, storage polysaccharides of amylolytic microbes; BA, amylolytic bacteria; BC, cellulolytic bacteria; HA, soluble rumen hexose originating from SC and ST; HC, soluble rumen hexose originating from NDF; MA, amylolytic microbes; PO, Protozoa. Other abbreviations as described for Figure 2A. Passage and absorption flows (see Figure 1) are not represented.

Table 2. Models and parameters

A; BA model		Parameter					
	$k_{lp,sp}$	f_{sp}	$k_{p,sp}$				
Model							
BA1	4.5	0.4	$k_{p,sol}$				
BA2	4.5	0.4	$k_{p,sol} \times (Q_{lp} + Q_{sp}) / Q_{sp}$				
BA3	8.5	0.6	$k_{p,sol} \times (Q_{lp} + Q_{sp}) / Q_{sp}$				
B; DA model		Parameter					
	c	$k_{p,s}$	$k_{p,m}$				
Model							
DA1	0.04	original values Danfær (1990)					
DA2	0.0006	$k_{p,liq} \times f_{liq} + k_{p,sol} \times (1 - f_{liq})$		$k_{p,liq}/2$			
DA3	0.04	$k_{p,liq} \times f_{liq} + k_{p,sol} \times (1 - f_{liq})$		$k_{p,liq}/2$			
DA4	0.4	$k_{p,liq} \times f_{liq} + k_{p,sol} \times (1 - f_{liq})$		$k_{p,liq}/2$			
C; DY model		Diet					
	1	2	3	4	5	6	7
Model parameter $T_{6.3}$							
DY1	17.0	14.0	15.5	0.0	20.0	15.0	18.0
DY2	9.5	8.5	8.5	0.0	6.0	5.0	6.0
Model parameter $T_{6.0}$							
DY1	5.0	4.5	4.5	0.0	4.0	2.0	4.0
DY2	8.0	7.0	7.0	0.0	5.0	4.5	5.0

Applied values for the unknown diet-specific parameters of fractional particle communication rate of Q_{lp} ($k_{lp,sp}$, /d), fraction of small particles in feed (f_{sp}) and fractional passage rate of Q_{sp} ($k_{p,sp}$, /d) in the BA model (with Q_{lp} and Q_{sp} as the quantity of large and small particles in the rumen, respectively), of the partition factor for carbohydrate fermentation (c) and fractional passage rate of substrate s ($k_{p,s}$, /d) and microbial matter ($k_{p,m}$, /d) in the DA model (with f_{liq} as the soluble fraction of substrate s in the diet), and of time interval of rumen pH below 6.3 and 6.0 ($T_{6.3}$ and $T_{6.0}$, respectively, h) in the DY model. Fractional passage rates $k_{p,sol}$ and $k_{p,liq}$ are observed values for rumen solids and fluid, respectively.

Table 3. Effect of input parameters on the BA, DA and DY models

Model	BA	DA	DY							
version	1	2 vs 1	3 vs 1	4 vs 2	1	2 vs 1				
Flow/ change of flow	(g/d)	%	%	%	(g/d)	%				
Duodenal flow										
TP	1762	15	-3	11	1802	-6	-4	-12	1829	2
NDF	1086	166	-48	38	1425	-14	0	-10	883	-13
TST	602	-1	13	9	105	0	52	260	379	3
TSC	36	36	-28	-2	49	226	6x10 ³	30x10 ³	344	0
Duodenal flow										
MP	1300	-20	31	4	1285	1	-6	-15	1259	4
MST	482	-20	31	4	99	-6	26	69	295	3
Mrest	491	-20	31	4	1214	-2	15	29	1117	4
Degraded										
P	1311	-39	26	11	2147	8	0	0	1507	0
NDF	4777	-39	61	-9	4439	5	0	3	4981	2
ST	221	-50	71	-17	468	0	-18	-28	322	0
Fermented										
P	1780	-28	26	-11	1015	26	-1	3	1408	-2
NDF	4191	-41	61	-12	2834	8	-1	15	3061	3
ST	306	-34	32	-14	457	0	-20	-45	297	-5
SC	2077	-3	0	-3	2377	-4	-9	-47	1367	-1

See Table 2 for clarification of model numbering. In the DA model 20% of microbial N was assumed to be non-amino acid N. Absolute value of version 1 and the percentage of change between the different versions of the BA, DA and DY models, averaged over seven diets, in simulated duodenal flow of total protein (TP), neutral detergent fibre (NDF), total starch (TST), total soluble carbohydrates (TSC), microbial protein (MP), microbial starch (MST), and microbial OM minus P and ST (Mres), in simulated amount of insoluble, degradable protein (P), NDF, starch (ST) degraded into soluble substrates, and in simulated amount of P, NDF, ST and soluble carbohydrates (SC) fermented into volatile fatty acids.

Results

BA model

Amendment of the parameter for fractional passage rate of Q_{sp} by multiplying $k_{p,sol}$ by $(Q_{lp}+Q_{sp}):Q_{sp}$ (BA2), increased duodenal flow of total P, NDF, total ST and SC, decreased substrate degradation and fermentation, and consequently decreased duodenal flow of microbial matter (BA2 vs. BA1; see Table 3 and Figures 3A, 4A, 5A & 6A). Although this amendment is theoretically correct with the use of observed $k_{p,sol}$ as a parameter input, the increased duodenal flows have to be considered as unrealistic since the predicted flows were far higher than those observed (Van Vuuren *et al.*, 1992, 1993). Therefore, simultaneously to the amendment of the parameter for fractional passage rate of Q_{sp} , parameters $k_{lp,sp}$ and f_{sp} also need to be altered.

Because duodenal flow of NDF is of 100% feed origin, it is the best output to illustrate the impact of parameters $k_{lp,sp}$ and f_{sp} on simulated particle dynamics. Sensitivity analysis (Figure 7) showed that very different combinations of $k_{lp,sp}$ and f_{sp} values can simulate a similar duodenal flow of NDF. Increased values of $k_{lp,sp}$ from 4.5 to 8.5 /d and of f_{sp} from 0.4 to 0.6, were considered most appropriate in attaining the observed duodenal flow of 0.7 kg of NDF /d (BA3) because this particular combination of parameter values simulated a majority of particles in Q_{sp} , corresponding to available evidence (Bosch *et al.*, 1992; Vaage, 1992). This change in parameter values improved predicted NDF flow for all diets: on average 50 and 26% of NDF intake in BA2 and BA3, respectively, compared to 24% observed (Van Vuuren *et al.*, 1992, 1993).

The change of $k_{lp,sp}$ and f_{sp} values in BA3 caused a different partitioning of OM between Q_{lp} , Q_{sp} and rumen fluid. Compared to BA1, rumen pool sizes (results not shown) in BA3 decreased but duodenal flows were comparable (BA3 vs. BA1 in Figures 3A, 4A, 5A & 6A). Compared with BA2, the Q_{lp} and Q_{sp} pools were markedly decreased and increased, respectively. Because of this, the proportion of microbes bound to Q_{sp} increased (see discussion BA model, below), which increased substrate degradation and fermentation, and thus duodenal flow of microbial matter (BA3 vs. BA2 in Table 3 and Figures 3A, 4A, 5A & 6A).

DA model

The use of observed passage rates as parameter input to the model (DA3) on average increased degradation and fermentation of P and NDF, decreased duodenal flow of P, NDF and microbial non-protein matter, and increased duodenal flow of SC (DA3 vs. DA1 in Table 3 and Figures 3B, 4B & 6B). Thus, the model is sensitive to fractional passage rates of fluid and solids. Changes were larger for some diets than for others, because Danfær's original estimates of fractional passage rates deviate more from the observed values for those diets.

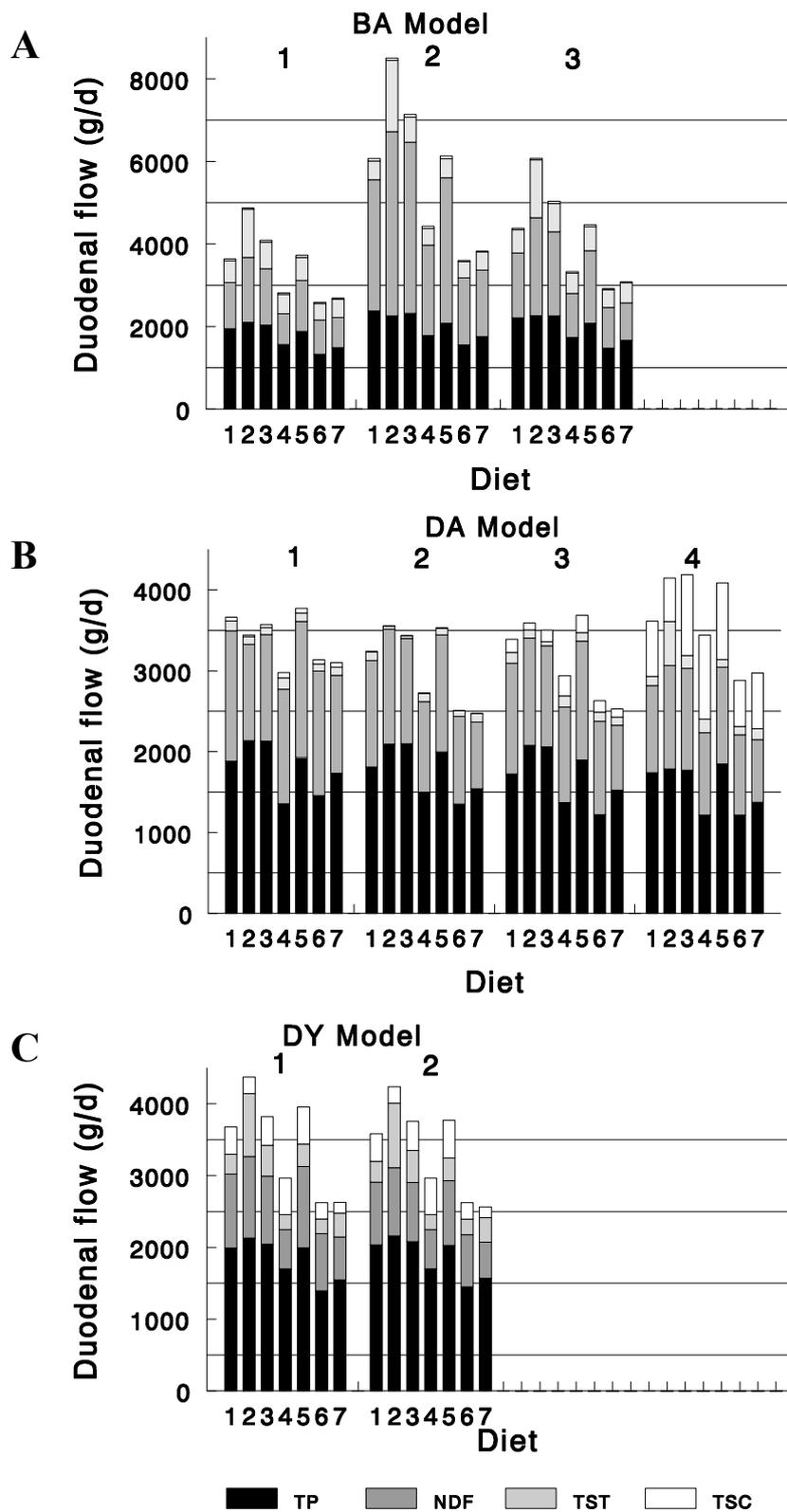


Figure 3. Simulated duodenal flow of protein and carbohydrates (g/d) in subsequent versions of the BA (A), DA(B) and DY(C) models. Abbreviations: TP, total protein; NDF, neutral detergent fibre; TST, total starch; TSC, total soluble carbohydrate.

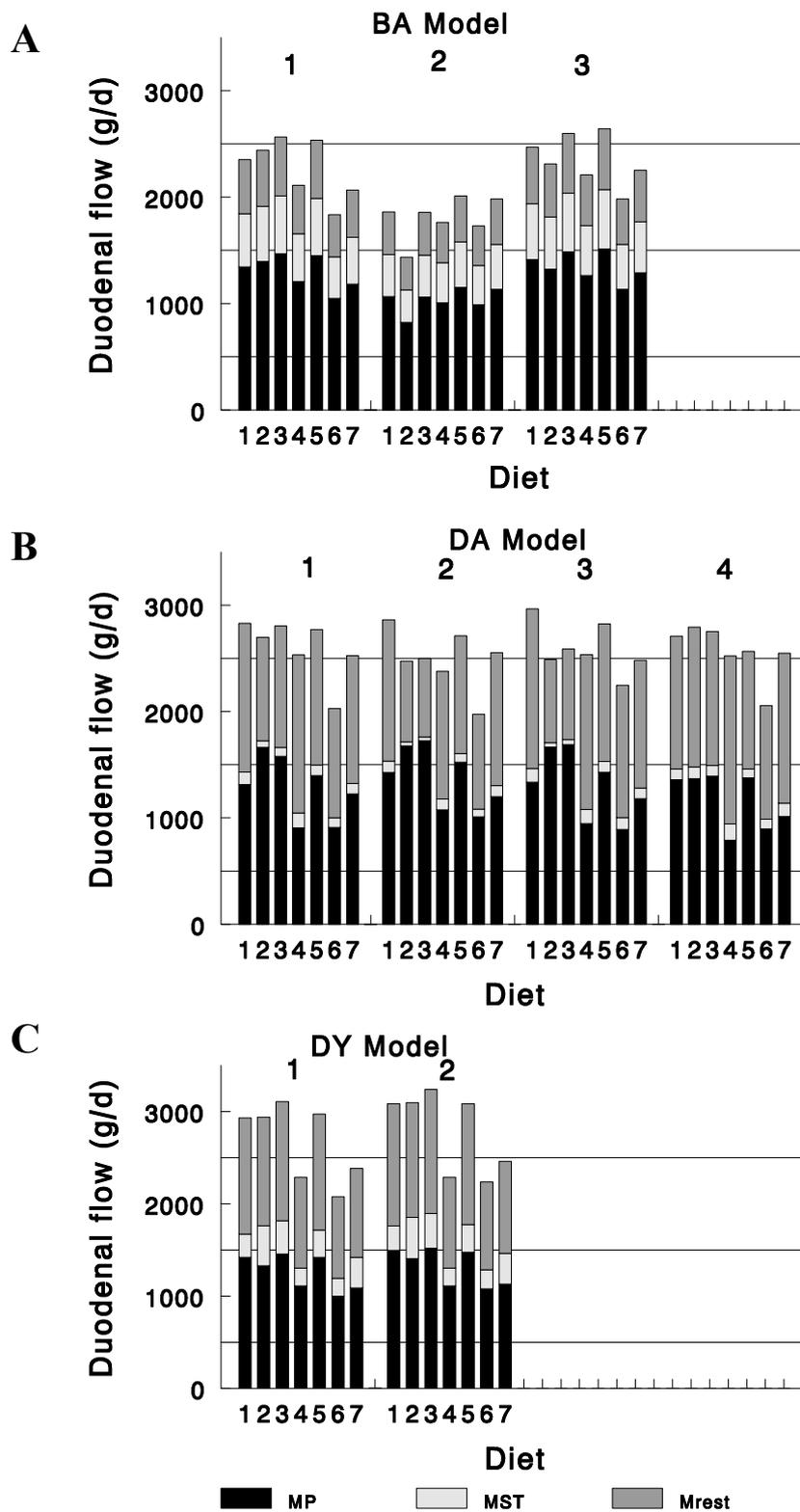


Figure 4. Simulated duodenal flow of microbial mass (g/d) in the subsequent versions of the BA (A), DA (B) and DY (C) models. Abbreviations: MP, microbial protein; MST, microbial starch; Mrest, microbial matter minus MP and MST.

With increasing values of parameter c for partitioning of the total carbohydrate fermentation into fermentation of SC, ST and NDF, the simulated fermentation of SC and ST decreased whereas that of NDF increased (in the order of DA2, DA3 and DA4; Table 3 and Figure 6B). In particular duodenal flows of SC and ST were influenced because their change in prediction is large compared to the predicted value with DA2. A higher c value redistributed total carbohydrate fermentation in the direction of more NDF fermentation instead of SC and ST fermentation, which delivered less ATP (Danfær, 1990). A lower ATP availability caused less microbial protein synthesis and less duodenal flow of microbial (and total) protein, which subsequently lowered carbohydrate fermentation (DA4 and DA3 vs. DA2 in Table 3 and Figures 2B, 3B, 4B & 5B). Less carbohydrate fermentation increased the total amount of carbohydrate present in the rumen ($Q_{sc}+Q_{st}+Q_{ndf}$), resulting in increased carbohydrate incorporation in microbial mass.

DY model

The impact of the changed values of the input parameters $T_{6,3}$ and $T_{6,0}$ in DY2 was small compared to the effects that were found on the behaviour of the BA and DA models (Table 3). Although a wide range of parameter estimates was applied in DY1 and DY2, simulation results remained similar (Table 2; Figures 3C, 4C, 5C & 6C). Except for the simulated amount of microbial NDF utilization and the remaining duodenal flow of NDF, changes in pH parameters had only minor effects on duodenal flow, the microbial contribution to duodenal flow, substrate degradation and substrate fermentation.

The highest $T_{6,0}$ values in DY2 (Table 2) caused the smallest fraction of protozoa in the amylolytic microbial pool and thereby the lowest predation on cellulolytic microbes. This effect in combination with the lowest reduction of cellulolytic activity (NDF degradation) with the smallest $T_{6,3}$ values (Table 2) resulted in the largest amount of NDF degradation and consequently the smallest amount of duodenal NDF flow with DY2 (DY2 vs. DY1 in Table 3 and Figures 3C & 5C). On average, simulated protozoal fractions in the pool of amylolytic microbes was 10% smaller in DY2 than in DY1. Less microbial recycling in DY2 stimulated the growth of cellulolytic microbes and consequently their outflow from the rumen (Table 3). Because of the high N content in the diets, the increased growth of cellulolytic bacteria did not inhibit growth of amylolytic microbes although both types of microbes use the same N sources. As a consequence, the increased cellulolytic microbial mass (5%) caused only a small reduction in the amylolytic microbial mass (-1%) and total microbial outflow increased (Table 3). Although similar amounts of dietary ST and P were degraded, the amount of ST and P fermented into VFA decreased because of a reduced microbial recycling (Figure 2C). The small impact of changed $T_{6,3}$ and $T_{6,0}$ does not imply that the simulated microbial recycling is equally small. On average, the simulated fraction protozoa in DY2 remained substantial with 27% of the amylolytic microbial pool, compared to 30% in DY1.

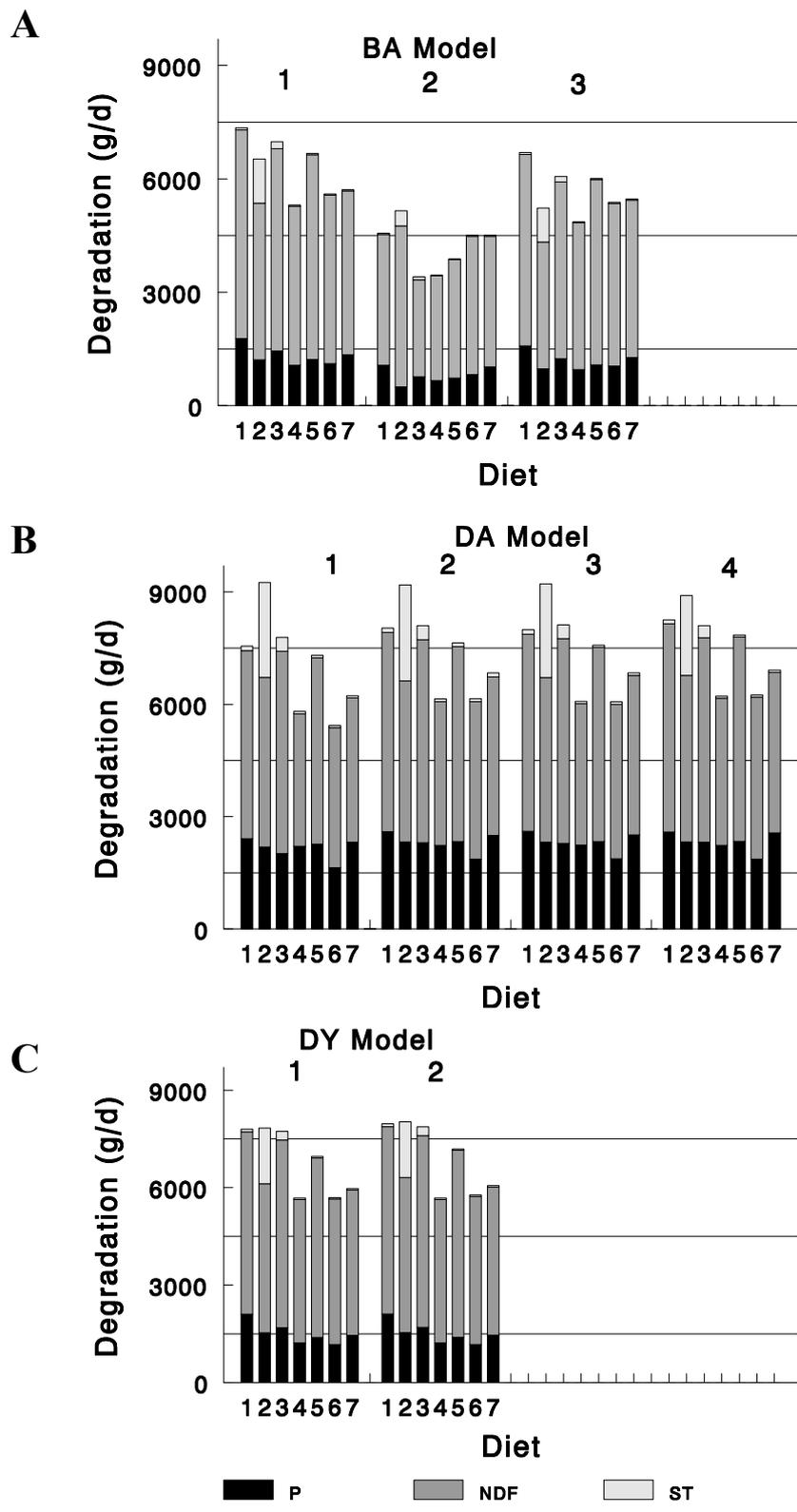


Figure 5. Simulated degradation of insoluble, degradable substrate into soluble substrate (g/d) in subsequent versions of the BA (A), DA (B) and DY (C) models. Abbreviations: P, protein; NDF, neutral detergent fibre; ST, starch.

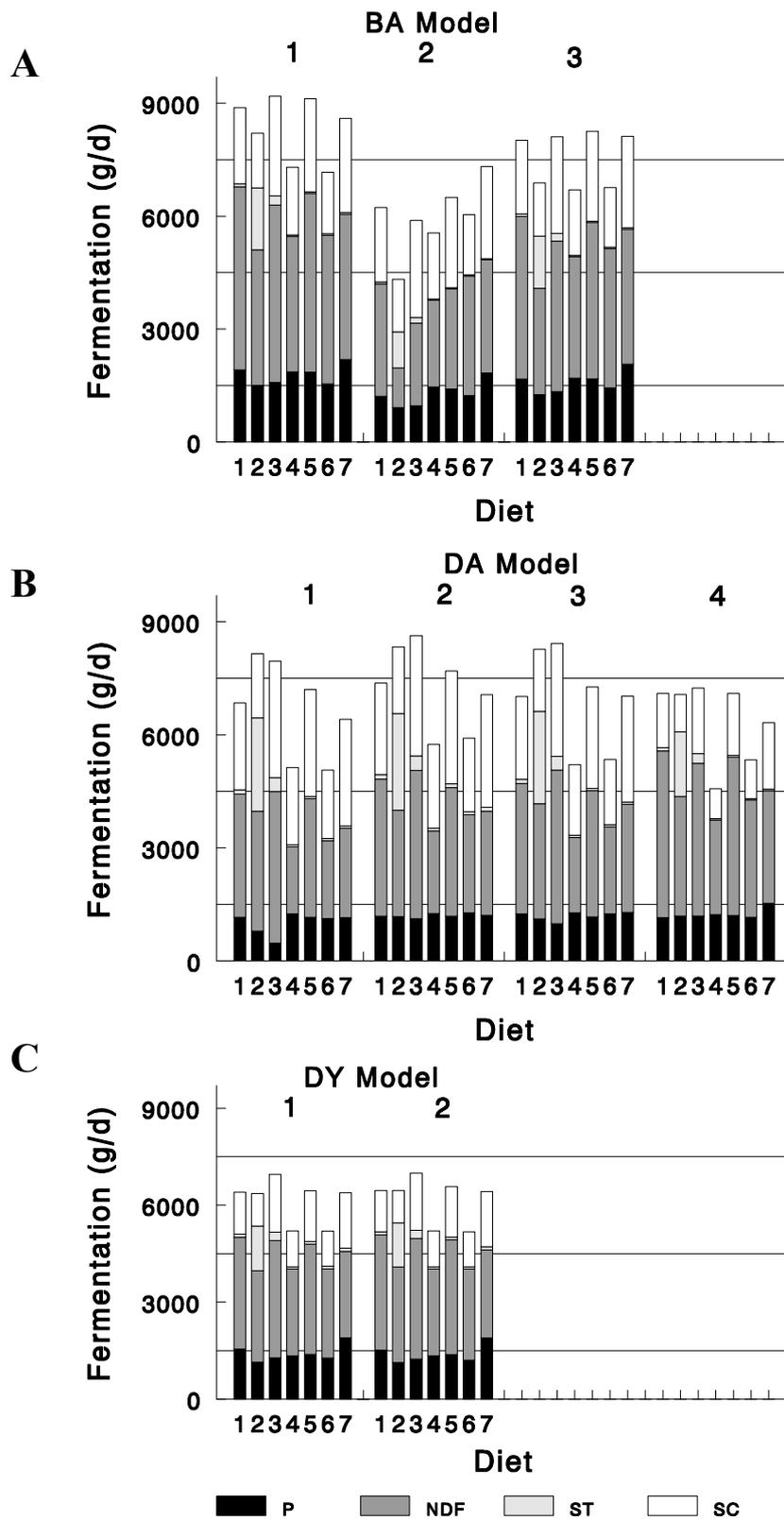


Figure 6. Simulated fermentation of substrate into VFA (g/d) in subsequent versions of the BA (A), DA (B) and DY (C) models. Abbreviations: P, protein; NDF, neutral detergent fibre; ST, starch; SC, soluble carbohydrates.

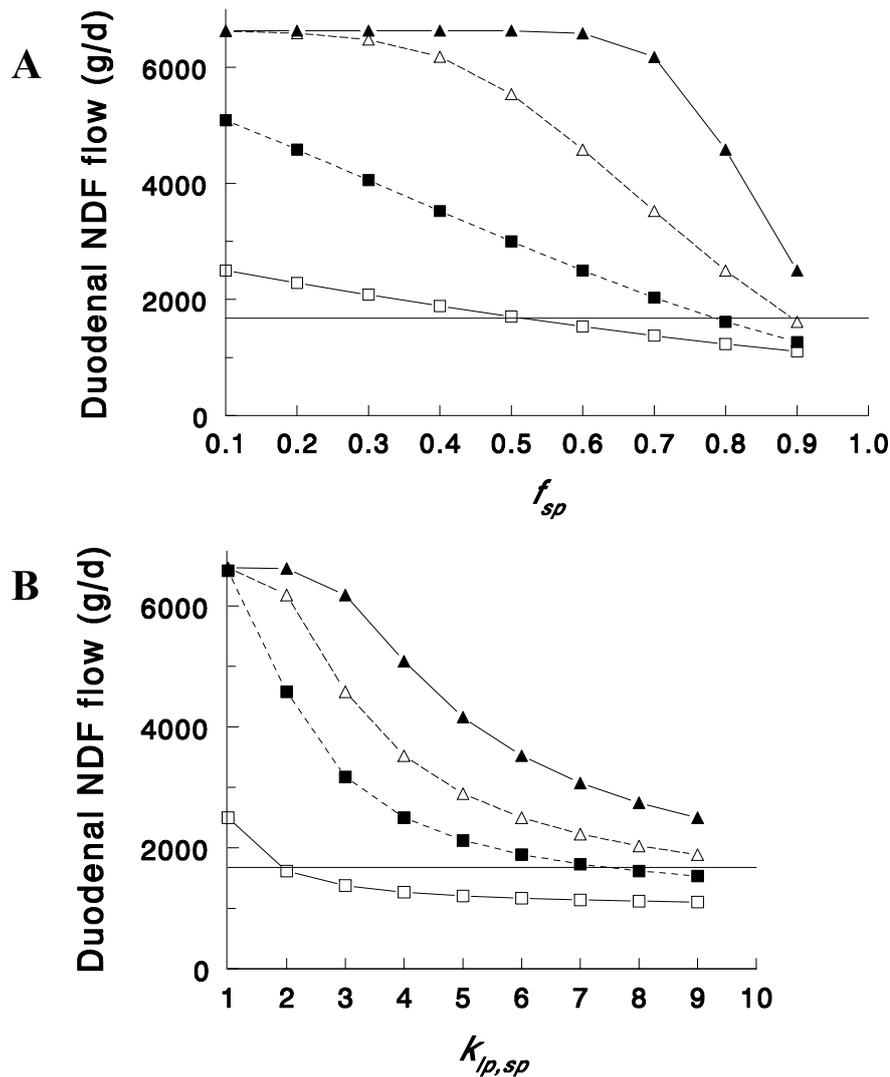


Figure 6. Effect of simultaneously varying the particle comminution rate ($k_{Ip,sp}$) from 1.0 to 9.0 /d and the particle size factor (f_{sp}) from 0.1 to 0.9 on simulated duodenal flow (g/d) of NDF (neutral detergent fibre). Simulations were performed using the BA2 model with inputs of diet 1 and the observed duodenal flow of NDF is indicated by the continuous line. (A) ▲, $k_{Ip,sp} = 1.0$; △, $k_{Ip,sp} = 2.0$; ■, $k_{Ip,sp} = 4.0$; □, $k_{Ip,sp} = 9.0$; (B) ▲, $f_{sp} = 0.1$; △, $f_{sp} = 0.4$; ■, $f_{sp} = 0.6$; □, $f_{sp} = 0.9$.

Discussion

Concepts and theories applied

Physical and metabolic factors are involved in the regulation of feed intake (Forbes, 1993). Physical factors are related to rumen pool sizes, comminution and passage rates, and rate of feed degradation. Metabolic factors include the level and pattern of nutrients (VFA, ammonia) absorbed from the rumen or ratios of absorbed nutrients. Rumen models can simulate the effect of dietary inputs on these factors and evaluate their importance in feed

intake regulation. The discussion will focus on the representation of these factors in rumen models, having in mind the various theories on intake regulation.

The models investigated in this study do not include a mechanism of intake regulation, but are more focused on description of the mechanism of chemical transactions in the rumen. They attempt to explain the interaction between feed characteristics and nutrients released from the rumen. The reason for using observed daily intake as an input to the models instead of predicting it, is obviously that no bias will be introduced due to wrong prediction of intake. Discontinuous inputs instead of constant inputs might result in different profiles of nutrients released from the rumen, although Robinson & Sniffen (1985) did not establish this *in vivo*. Rumen models have so far not been used for simulation of the effect of discontinuous inputs, except for the early model of France *et al.* (1982). Because the published results of observations of rumen digestion are almost always limited to calculated mean daily pool sizes and flows, models are only evaluated on their simulation to steady-state with constant inputs (Baldwin *et al.*, 1987; Neal *et al.*, 1992). When evaluating their steady-state behaviour with observed discontinuous inputs on the observed daily pattern, instead of the daily means of rumen pool sizes and flows, more detailed information might be gathered on prediction inaccuracies and faults in the model descriptions. Moreover, when future improvements of the rumen models are to be applied in the practice of ruminant nutrition, theories on factors that regulate daily intake as investigated by Poppi *et al.* (1994) need to be included because daily intake is an important but unpredictable factor in ruminant nutrition (De Visser, 1993).

The representation of microbial activity is the major determinant in simulated rumen function. However, the concepts applied in the investigated models differ widely. In the BA model separate state variables are used for microbes attached to large particles, to small particles, to insoluble, degradable ST, to insoluble, degradable NDF, and suspended free in rumen fluid (Figure 2A). The DA model distinguishes one type of microbial growth but separate state variables for the microbial content of free ammonia, of free amino acids, of microbial protein, and of combined microbial lipid, starch and cell wall with a fixed composition (Figure 2B). Finally, the DY model distinguishes state variables for cellulolytic bacteria (growing on NDF), amylolytic microbes (growing on SC and ST) and a storage pool of polysaccharides in amylolytic microbes (Figure 2C). The last two amylolytic state variables are both subdivided in a fixed bacterial and protozoal fraction (protozoal fraction estimated from feed characteristics). Thus, even the basic elements in the formulated microbial mechanism are totally different and large differences are simulated in the amounts of insoluble, degradable substrate degraded into soluble substrate (Figure 5) and substrate fermented into VFA (Figure 6) or incorporated in microbial matter (Figure 4), and substrate passing to the duodenum (Figure 3). Evaluation of the models on the same observed input/output data will enable a model comparison and a test of the proposed mechanisms and theories applied, without being confounded by differences in observed datasets used. Model evaluation is however not the goal of this paper. Instead, the goal is to demonstrate the impact

of diet-specific parameters on model behaviour and the differences in applied theories and concepts that are related to these parameters. Each model will be discussed now, simultaneously with its differences to the other models.

BA model

The DA and DY models mainly describe the chemical transactions in the rumen compartment and physical aspects of rumen function are solely described by the parameter inputs of fluid and solid passage rates. The BA model differs in that it integrates the physical concept of particle dynamics with the chemical transactions (Figure 2A). There is abundant evidence for such a mechanism in literature (Czerkawski, 1986; Faichney, 1993; Kennedy & Murphy, 1988; Ulyatt *et al.*, 1986). Although inclusion of multiple particle pools enables a realistic delay for the availability of ingested feed, because large particles first have to be comminuted to small particles before becoming available for microbial use, it still has to be fully parameterized by (two) input parameters, like the DA and DY models. However, it is not clear how the parameter values change with changes in diets or how to estimate them. The major advantage of representing a mechanism of particle dynamics is that the introduced delay creates the possibility of simulating duodenal flows as well as rumen pool sizes. This feature is of particular importance when physical factors involved in intake regulation (Forbes, 1993) are to be investigated.

The BA model also describes specific microbial growth on ST or on NDF with a mechanism for the physical interactions between microbes and small particles. The total microbial mass in the rumen is assumed to be distributed among large particle mass, small particle mass and rumen fluid in the same ratio as the occurrence of OM in these three physical compartments (MLP, MSP and MFLUID in Figure 2A). The specific growth is described with separate state variables of microbes that are specifically bound to ST small particles on the one hand and microbes that are specifically bound to NDF small particles on the other hand. These microbes degrade and grow on the type of particles they are attached to (MST and MNDF in Figure 2A). Except for rumen pH (Argyle & Baldwin, 1988), no parameter inputs are required to parameterize substrate degradation and the model is thus rather self-explanatory. However, it is debatable whether representation of the effect of rumen pH (only on NDF degradation) and particle dynamics is sufficient to explain observed variation in feed degradation characteristics (Nocek & Russell, 1988; Tamminga *et al.*, 1990). It is unlikely that changes in parameterization of the mechanism of particle dynamics and pH only can deliver a similar variation in degradation characteristics of feeds as observed. This is the reason for using observed parameters in the DY model which will be discussed. To what extent the BA model is capable of explaining observed variation in degradation characteristics remains to be established.

DA model

In the DA model it was not clearly stated whether the considerable number of distinct parameters of passage rate were model inputs or not. If they were considered variable and therefore dependent on the diet, as in the BA and DY models, it remains obscure how they should be estimated or what concepts apply to these parameters. Although the concept of fluid and solid passage from the rumen is generally regarded as essential to understanding rumen function (Owens & Goetsch, 1986; Faichney, 1993), this was apparently not used in the DA model. As a consequence, rumen state variables were also not separated into those that are affected by fluid passage rate and those by solid passage rate (Figure 2B).

As for the BA model, the DA model also does not use parameters of feed degradation characteristics other than the fraction of protein and NDF that is undegradable. Furthermore, the model does not describe a mechanism of specific microbial growth on ST or NDF, or include the effect of rumen pH. The relationships containing the c parameter in itself do not affect changes in microbial growth or fermentation rate. However, varying c will cause a slightly different ATP yield because the assumed ATP yields are slightly different for SC, ST and NDF, and consequently slightly alter microbial growth. However, this seems rather limited for explaining observed variation in feed degradation (Nocek & Russell, 1988; Tamminga *et al.*, 1990) and microbial growth efficiencies (Owens & Goetsch, 1986). In the DA model the unique concept is applied of one type of microbe represented with separate state variables (MAM, MPS, Mp and MC in Figure 2B) of four microbial nitrogenous constituents and one for the remaining non-nitrogenous constituents (starch, lipid, cell wall). Thus, the composition of microbial mass and the stoichiometry of the synthesis of microbial mass can vary. This is in contrast to the BA and DY models that define separate state variables for distinct types of microbes and apply a fixed stoichiometry for synthesis of total and non-polysaccharide microbial matter from substrate, respectively (they do not apply a fixed total requirement for microbial growth however). This study indicates that the representation of microbial function in the DA model does result in more variation in the composition of microbial mass over diets than with the BA and DY models (Table 3 and Figure 4). In the DA model however, the polysaccharide or starch content of the state variable of non-protein microbial mass (MC in Figure 2B) was assumed to be fixed, although this fraction is generally thought to be the most variable fraction in microbial contents (Czerkawski, 1986). Moreover, with low c values in DA2 and DA3 the microbial starch content even seems to decrease with diets 2 and 3 that contain more ST or SC, which is unlikely to occur *in vivo*, whereas the highest c value in DA4 diminished the variation over diets in simulated microbial composition (Figure 4).

DY model

The microbial metabolism itself is described in most detail in the DY model with separate relationships for growth, storage of polysaccharides, substrate fermentation and

passage of each type of microbial mass distinguished. cellulolytic bacteria, amylolytic bacteria and protozoa, amylolytic storage polysaccharides (BC, BA, PO and AS in Figure 2C). Moreover, the DY model describes protozoal predation on cellulolytic bacteria and protozoal death, and thus it is the only model that describes intra-ruminal recycling. There is abundant evidence for this recycling to occur in significant magnitude in the rumen (Williams & Coleman, 1992). In a more comprehensive version of the model (Dijkstra, 1994b), the protozoal functions are described in more detail with independent state variables for amylolytic bacteria and protozoa instead of the fixed ratio of protozoal and amylolytic bacterial mass in the DY model. In contrast with the BA and DA models, the authors describe in detail how individual model relationships were derived from literature, which makes their applied concepts and theories fully accessible to others.

The model requires parameter inputs for substrate degradation rate that are derived from routine measurements of feed degradation rate in nylon bags that have been placed inside the rumen *in vivo* through a rumen cannula. Thus, the model receives differences in substrate degradation as an input instead of leaving the explanation of these differences to the model, as in the case of the BA and DA models (Baldwin, 1995). Moreover, the methodology used with nylon bag studies has a large impact on the results obtained (Lindberg, 1985; Nocek, 1988; Van der Koelen *et al.*, 1992) and results can deviate systematically from the *in vivo* situation. However, the DY model describes substrate degradation by comparing these parameter inputs to reference values that were measured with similar techniques. This approach reduces the chance of introducing large systematic errors due to the application of these inputs. The advantage is that the results of nylon bag studies appropriately reflect the specific degradation characteristics of a feed. With no input on degradation characteristics at all, models probably remain incapable of explaining observed differences in feed degradation. The wide range of pH intervals tested in this study had a small effect on simulated substrate degradation (Figure 5C). Whether the use of direct observations on feed degradation characteristics results in an improved simulation of rumen function remains to be established. As far as the authors know, extensive model evaluations on a wide range of dietary inputs have only been published for the DY model (Neal *et al.*, 1992) and thus cannot be compared with the BA and DA models.

Where the DY model requires parameter inputs (besides feed intake and composition) of observed degradation characteristics of feeds, the BA and DA models require other parameter inputs that also indirectly affect simulated substrate degradation. These inputs have been declared diet-dependent and thus are variable inputs (Baldwin, 1995; Danfær, 1990). In the BA model, feed degradation, feed passage, microbial passage, microbial growth and specific microbial growth on ST or on NDF, are strongly dependent on the mechanism of particle dynamics. Thus the estimates made for the $k_{lp,sp}$ and f_{sp} parameters in this mechanism markedly affect simulated substrate degradation (Figure 5A). In the DA model, the c

parameter had small effects on substrate degradation (Figure 5B), despite the large effect on the simulated duodenal flow of feed carbohydrates (Figure 3B).

Practicability of diet-specific parameters

The diet-specific parameters that are required as an input in addition to feed composition and intake adapt the models to the rumen conditions that are specific for that type of diet. This is a convenient way of introducing the complex regulation of rumen condition into the models, without having a description of the mechanism to explain them. However, the parameter inputs investigated in this study are difficult to estimate. This will limit the application of the models to the evaluation of feeds in current ruminant production systems. Therefore, all the rumen models are considered to be research models that can be useful in testing hypothesis and developing theories. The DY model uses the most parameter inputs to account for the variation in rumen condition (degradation characteristics of feed in nylon bags inside the rumen, rumen liquid volume, liquid and solid fractional passage rates estimated from sampling the flow of specific markers that have been introduced into the rumen, and the daily pattern of rumen pH). Consequently, more input parameters are needed to run the DY model than to run the DA or the BA models. But, the concepts of the applied parameter inputs are readily applicable to observations currently made on rumen digestion, whereas the parameter inputs for particle dynamics in the BA model and for partitioning of carbohydrate fermentation in the DA model rely much more on the judgement of the model user. For this reason, practicability for evaluation of feeds seems highest for the DY model.

Importance of rumen passage

The inadequate representation of particle dynamics and passage rates (in particular that of solids) in rumen models is generally recognized (Kennedy & Murphy, 1988; Faichney, 1993). Van Soest *et al.* (1988) stated that models published previously to those investigated in this study were limited in their description of particle dynamics. This qualification still holds for the DA and DY models, and to a lesser extent also for the BA model because estimates are not measured or it is unclear how appropriate estimates should be obtained. The BA model adopts the hypothesis that large particles have to be comminuted to a small size and only these small particles can leave the rumen. However, recent evidence indicates this hypothesis is not universally applicable and still is a major simplification of rumen passage processes. It has been found that a large proportion of the rumen particles is below the critical particle size threshold and should be available for passage, but other factors prevent these small particles actually leaving the rumen (Kennedy & Murphy, 1988). These factors include the buoyancy and functional specific gravity of particles (Kaske *et al.*, 1992) and need to be represented in the parameter inputs to the BA model to describe rumen particle dynamics. However, it is unlikely that the mechanism of particle dynamics in the BA model can accommodate such effects because there is no method to estimate the appropriate parameter values. Thus, a larger

number (Beever *et al.*, 1986) and different types of particle pools may be required to represent rumen particle dynamics. Varying the values of passage rate parameters has a significant influence on simulated rumen pool sizes and flows (physical factors which might be involved in the regulation of feed intake) and therefore the topic of estimating appropriate values is not a trivial problem. Application of passage rates that are measured with experimental methods or under conditions that do not correspond to those on which the model is calibrated, will seriously affect simulation results. In particular, the description of the mechanism that regulates the passage needs improvement. Incorporation of a more mechanistic explanation of particle dynamics would result in a better understanding of rumen function. In this respect, the mechanism of particle dynamics included in the BA model is a good example.

Concluding remarks

It can be concluded that the BA, DA and DY models emphasize different aspects of microbial function in the rumen with their own required parameter inputs. Therefore, arguments about a specific advantage or disadvantage of one model often also holds for the other models but on different concepts and theories that have been represented herein. The BA model gives the best opportunity for adequate simulation of both rumen pool sizes and flows because of the mechanism of particle dynamics it includes. On the other hand, the DY model only requires parameter inputs whose concepts correspond to current rumen digestion measurements, but it is limited in describing rumen particle dynamics. Moreover, it gives the most comprehensive integration of current theories in the functioning of rumen microbes. The DA model (as published) is most limited in describing particle dynamics and specific microbial growth on ST or on NDF. Although unique concepts are used for the representation of rumen microbes, there is no apparent advantage in this.

Evaluation of alternative mechanisms of microbial function on the same independent observations could give important additional information to improve the models. Comparison of simulation results in this study reveals a clear distinction between the models in the basic microbial functions of substrate degradation, substrate fermentation into VFA, microbial growth and microbial outflow. This has a large influence on the predicted profile of nutrients released from the rumen. The VFA in particular are important because they constitute up to two-thirds of the energy in nutrients absorbed from the gastro-intestinal tract (Sutton, 1985). The large differences between models in the simulated amount of substrate fermented into VFA indicates that simulated VFA production and molar proportions of acetate, propionate and butyrate in particular need further evaluation. It has already been established that molar proportions of rumen VFA are predicted inaccurately (Baldwin *et al.*, 1987; Neal *et al.*, 1992). To investigate theories that go beyond the rumen, a correct simulation of the profile of absorbed nutrients is required. In particular the regulation of feed intake is of interest if these models are to become useful in practice. It has been postulated that the total amount as well as the diurnal patterns of absorbed nutrients are important factors in the regulation of daily feed

intake (Gill & Romney, 1994; Poppi *et al.*, 1994). At present, the models are still fully oriented to describing the chemical transactions in the rumen compartment to obtain a better explanation of the profile of nutrients that is released from the rumen per day.

In this study, it was shown that the manipulation of the diet-specific parameter inputs has a large impact on model behaviour, which is to be expected with parameters that are declared to be dependent on the diet. But, for most of these inputs it is uncertain how they should be estimated, and they do not always relate to observations. Future modelling efforts should thus also be directed at further refinement of the mechanisms in extant models that cannot readily be parameterized, such as the mechanisms of particle dynamics and passage rates.

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References

- Argyle, J.L. & Baldwin, R.L., 1988. Modelling of rumen water kinetics and effects of rumen pH changes. *Journal of Dairy Science* 71, 1178-1188.
- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54, 107-131.
- Baldwin, R.L., 1995. *Modeling Ruminant Digestion and Metabolism*. Chapman & Hall, London, United Kingdom.
- Beever, D.E., France, J. & Theodorou, M.K., 1986. Modelling of rumen function. In: A. Neimann-Sørensen (Ed.), *New Developments and Future Perspectives in Research on Rumen Function*, EEC, Brussels, pp. 109-140.
- Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70, 567-590.
- Bosch, M.W., Lammers-Wienhoven, S.C.W., Bangma, G.A. & Van Adrichem, P.W.M., 1992. Influence of stage of maturity of grass silages on digestion processes in dairy cows. 2. Rumen contents, passage rates, distribution of rumen and faecal particles and mastication activity. *Livestock Production Science* 32, 265-281.
- Czerkawski, J.W., 1986. *An Introduction to Rumen Studies*. Pergamon Press, Oxford, United Kingdom.
- Danfær, A., 1990. *A Dynamic Model of Nutrient Digestion and Metabolism in Lactating Dairy Cows*. Ph.D. Thesis, Report 671, National Institute of Animal Science, Denmark.
- De Visser, H., 1993. *Influence of Carbohydrates on Feed Intake, Rumen Fermentation and Milk Performance in High-yielding Dairy Cows*. PhD Thesis, Wageningen Agricultural University, The Netherlands.
- Dijkstra, J., Neal, H.D.StC., Beever, D.E. & France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen. Model description. *Journal of Nutrition* 122, 2239-2256.
- Dijkstra, J., 1994a. Production and absorption of volatile fatty acids in the rumen. *Livestock Production Science* 39, 61-69.
- Dijkstra, J., 1994b. Simulation of the dynamics of protozoa in the rumen. *British Journal of Nutrition* 72, 679-699.

- Faichney, G.J., 1993. Digesta flow. In: J.M. Forbes & J. France (Eds.), *Quantitative Aspects of Ruminant Digestion and Metabolism*, CAB International, Wallingford, United Kingdom, pp. 53-85.
- France, J., Thornley, J.H.M. & Beever, D.E., 1982. A mathematical model of the rumen. *Journal of Agricultural Science, Cambridge* 99, 343-353.
- Forbes, J.M., 1993. Voluntary feed intake. In: J.M. Forbes & J. France (Eds.), *Quantitative Aspects of Ruminant Digestion and Metabolism*, CAB International, Wallingford, United Kingdom, pp. 479-494.
- Gill, M. & Romney, D., 1994. The relationship between the control of meal size and the control of daily intake in ruminants. *Livestock Production Science* 39, 13-18.
- Kaske, M., Hatiboglu, S. & Engelhardt, W.V., 1992. The influence of density and size of particles on rumination and passage from the reticulo-rumen of sheep. *British Journal of Nutrition* 67, 235-244.
- Kennedy, P.M. & Murphy, M.R., 1988. The nutritional implications of differential passage of particles through the ruminant alimentary tract. *Nutrition Research Reviews* 1, 189-208.
- Lindberg, J.E., 1985. Estimation of rumen degradability of feed proteins with the in sacco technique and various in vitro methods. *Acta Agriculturae Scandinavica* 25 (Supplement), 64-97.
- Neal, H.D.StC., Dijkstra, J. & Gill, M., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen. Model evaluation. *Journal of Nutrition* 122, 2257-2272.
- Nocek, J.E., 1988. In situ and other methods to estimate ruminal protein and energy digestibility. A review. *Journal of Dairy Science* 69, 77-87.
- Nocek, J.E. & Russell, J.B., 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *Journal of Dairy Science* 71, 2070-2107.
- Owens, F.N. & Goetsch, A.L. 1986. Digesta passage and microbial protein synthesis. In: L.P. Milligan, W.L. Grovum & A. Dobson (Eds.), *Control of Digestion and Metabolism in Ruminants*, Prentice-Hall, Englewood Cliffs, NJ, pp. 196-222.
- Poppi, D.P., Gill, M. & France, J., 1994. Integration of theories on intake regulation in growing ruminants. *Journal of Theoretical Biology* 167, 129-145.
- Robinson, P.H. & Sniffen, C.J., 1985. Forestomach and whole tract digestibility for lactating dairy cows as influenced by feeding frequency. *Journal of Dairy Science* 68, 857-867.
- Speckhart, F.H. & Green, W.L., 1976. *A Guide to Using CSMP-The Continuous System Modelling Program*. Prentice-Hall, Englewood Cliffs, NJ.
- Sutton, J.D., 1985. Digestion and absorption of energy substrates in the lactating cow. *Journal of Dairy Science* 68, 3376-3393.
- Tamminga, S., Van Vuuren, A.M., Van der Koelen, C.J. & Van der Togt, P.L., 1990. Ruminal behaviour of structural carbohydrates, non-structural carbohydrates and crude protein

- from concentrate ingredients in dairy cows. *Netherlands Journal of Agricultural Science* 38, 513-526.
- Ulyatt, M.J., Dellow, D.W., John, A., Reid, C.W.S. & Waghorn, G.C., 1986. Contribution of chewing during eating and rumination to the clearance of digesta from the ruminoreticulum. In: J.P. Milligan, W.L. Grovum & A. Dobson (Eds.), *Control of Digestion and Metabolism in Ruminants*, Prentice-Hall, Englewood Cliffs, NJ, pp. 498-515.
- Vaage, A.S., 1992. *Control of Particle Outflow from the Reticulo-rumen*. PhD Thesis, University of Guelph, Canada.
- Van der Koelen, C.J., Goedhart, P.W., Van Vuuren, A.M. & Savoni, G., 1992. Sources of variation of the in situ nylon bag technique. *Animal Feed Science of Technology* 38, 35-42.
- Van Soest, P.J., Sniffen, C.J. & Allen, M.S., 1988. Rumen dynamics. In: A. Dobson & M.J. Dobson (Eds.), *Aspects of Digestive Physiology in Ruminants*, Prentice-Hall, Englewood Cliffs, NJ, pp. 21-42.
- Van Vuuren, A.M., Krol-Kramer, F., Van der Lee, R.A. & Corbijn, H., 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. *Journal of Dairy Science* 75, 2215-2225.
- Van Vuuren, A.M., Van der Koelen, C.J. & Vroon-de Bruin, J., 1993. Ryegrass versus corn starch or beet pulp fibre diet effects on digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science* 76, 2692-2700.
- Williams, A.G. & Coleman, G.S., 1992. Role of protozoa in the rumen. In: A.G. Williams & G.S. Coleman (Eds.), *The Rumen Protozoa*, Springer-Verlag, New York, pp. 317-347.

Chapter 3

Comparison of Mechanistic Rumen Models on Mathematical Formulation of Extra-microbial and Microbial Processes

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Abstract

This study investigated the consequences of differences in applied concepts and individual mathematical formulations on steady-state behaviour of three important mechanistic rumen models. In the models of Baldwin *et al.*, (1987) and Danfær (1990), the formulation of passage rate, non-dietary inputs, defined rumen substrate pools, absorption rates, degradation rates, molecular weights, parameterization of VFA production, and physical compartmentalization were sequentially exchanged for the formulation of the model of Dijkstra *et al.*, (1992). Most of these adaptations had a considerable influence on model behaviour, indicating large qualitative differences in formulation and sensitivity to concept choice. Because microbial substrate environments were similar after all adaptations, the microbial mechanisms could be compared objectively without being concealed by differences in extra-microbial formulation. None of the microbial functions were altered except for substrate degradation, which gave rise to a similar rate of substrate entrance to soluble rumen pools that are available for microbial utilization. Large differences remained in microbial functions of substrate fermentation, substrate incorporation, and microbial synthesis. Differences in extra-microbial rumen functions and microbial mechanisms had important consequences for simulated nutrient outputs from the rumen, illustrating the necessity for further validation of individual formulations.

Introduction

The rumen digestion of feed largely determines which nutrients are absorbed from the gastrointestinal tract. Mathematical models have been constructed (Dijkstra, 1993; Gill *et al.*, 1989; Thornley & France, 1984) to integrate the acquired knowledge of rumen processes. To our knowledge, an extensive comparison of existing models has not been attempted. Therefore, we performed a comparative study on three recently published mechanistic models of rumen function by Argyle & Baldwin (1988) and Baldwin *et al.* (1987; BA), Danfær (1990; DA), and Dijkstra *et al.* (1992; DY). Although these models are broadly similar in concept, a detailed inspection demonstrates many differences in individual mathematical equations. Because many differences exist simultaneously in mathematical formulation, it is unclear to what extent distinct model behaviour must be ascribed to a specific mathematical equation. An option to resolve differences between formulations could be the comparison and analysis of the fundamental data and the justification for the alternative mathematical equations of each model. However, we compared individual formulations within the context

of the models, which enabled us to interpret the consequences of a specific formulation for total model behaviour.

Concepts and formulations were made similar for each model by sequential adaptation of individual formulations or concepts and analysis of the resulting effect on model behaviour. This approach can be regarded as a behavioural analysis of alternative mathematical formulations. An additional objective was to create a similar microbial substrate environment after all adaptations (i.e., equal rates of substrate becoming available for microbial utilization). Remaining differences in flows and pool sizes could then only be ascribed to differences in formulation of the microbial mechanisms, which enabled an objective comparison of the mathematical formulation of the central microbial mechanism, without additional differences in formulation of the extra-microbial rumen processes.

Materials and methods

Selected Rumen Models and Model Inputs

Three recently published mechanistic rumen models were selected that formulated the microbial mechanism following widely differing concepts: the rumen part of the BA, the rumen part of the DA, and the DY. An updated version (Argyle & Baldwin, 1988) of the BA was used with a pH dependency of VFA production, NDF degradation, and microbial maintenance requirement, an additional input of urea absorption into the rumen, and a halved urea concentration of blood plasma. The BA and DY were translated into CSMP (Speckhart & Green, 1976). Simulations were performed with a fourth-order variable step length Runge-Kutta as integration method and integration periods for the BA, DA, and DY for 20, 50, and 20 d, respectively. Models were compared for their steady-state behaviour.

Model simulations were performed with constant mean daily inputs derived from Van Vuuren *et al.*, (1992, 1993); a ryegrass diet.(diet 1; Van Vuuren *et al.*, 1993), partially replaced by concentrate mixtures based on corn starch (diet 2; Van Vuuren *et al.*, 1993) or sugar beet pulp fibre (diet 3; Van Vuuren *et al.*, 1993); and ryegrass harvested with low (diets 5 and 6; Van Vuuren *et al.*, 1992) or high (diets 4 and 7; Van Vuuren *et al.*, 1992) rates of N fertilization, harvested in summer (diet 4 and 5; Van Vuuren *et al.*, 1992) or fall (diets 6 and 7; Van Vuuren *et al.*, 1992). These seven diets demonstrated moderate contrasts in chemical composition (soluble carbohydrate, starch, NDF, and N), degradability (*in situ* estimates of the soluble fraction, the insoluble, degradable fraction, the insoluble, undegradable fraction, and the fractional degradation rates of the insoluble, degradable fraction of starch, NDF, and N), rumen volume, fractional passage rates, and rumen pH (Table 1).The original models were used as published with a fractional particle comminution rate and particle size factor of 4.5/d and 0.4, respectively (Table 1), experimental fluid and OM fractional passage rates, and experimental rumen volume as input parameters in the BA; a parameter value of 0.04 in the

equations determining the partitioning of total carbohydrate fermentation into sugar, starch, and NDF fermentation (Table 1; Equations [10] through [12] in Table A4), and experimental rumen volume as input parameters in the DA; and experimental intervals for pH <6.3 and 6.0 (Table 1), experimental fluid and OM fractional passage rates, experimental rumen volume, and *in situ* starch, NDF, and protein degradation rates as input parameters in the DY. The soluble; the insoluble, degradable; and the insoluble, undegradable fractions of dietary inputs were derived from *in situ* results. Table 1 describes the required dietary and parameter inputs for the BA, DA, and DY.

Model Adaptations

The DY was chosen as the reference model because inclusion of its formulations and concepts in the other models required the least programming work. Different formulations of extra-microbial rumen functions were identified and, to eliminate differences between models, nine adaptations were made to the BA and DA (Table 2). Adaptations were performed sequentially; each subsequent model version involved an additional adaptation. Numbered model indications refer to a specific simulation, and no numbering means model descriptions were as published. Because some adaptations were unnecessary in the BA and DA, certain model numbers are missing (Table 2). The sequence of adaptations was subsequently directed at extra-microbial aspects of rumen function (passage rates, non-dietary substrate inputs, absorption rates, and distinguished rumen substrate pools), the microbial mechanism (substrate degradation, degradable substrate fractions, and molecular weights of substrate toward substrate availability from ingested DM, and VFA production), and the differentiation in rumen particle pools. Changes in mathematical equations and parameter values with these adaptations are summarized in Tables A1 through A7 in the Appendix, which also enables a direct comparison between models.

Experimental passage rates in the DA. The original DA (DA0) contained constant fractional passage rates, independent of experimental conditions. As in the DY, experimental estimates were used as passage rates in the DA1. Experimental estimates of fractional liquid (kp_{liq}) and OM passage rate (kp_{OM}) were assumed to contribute to the fractional passage rate of rumen substrate pools (kp_{sub}) in proportion to the input fractions of soluble (fs) and insoluble substrates ($1 - fs$). The kp_{sub} is then given by the equation: $fs \times kp_{liq} + (1 - fs) \times kp_{OM}$. Microbial fractional passage rates were chosen to be one-half that of rumen fluid.

Urea transfer to the rumen and saliva input. The BA and DY allow for non-dietary input of urea and protein into the rumen (Table A2), but the DA only accounts for urea transfer into the rumen. Inclusion of the equations of Dijkstra *et al.* (1992) for protein input with saliva production and urea transfer across the rumen wall provided mathematically identical formulation for non-dietary inputs in the BA2 and DA2.

Table 1. Model inputs ¹ for the models of Baldwin *et al.* (1987; BA), Danfær (1990; DA), and Dijkstra *et al.* (1992; DY).

	Diet ²							Model
	1	2	3	4	5	6	7	
Chemical composition								
(g/kg product)								
DM	885	912	835	878	889	872	874	...
(g/kg of DM)								
SC	146	104	110	164	174	146	193	BA DA DY
DST ³	6	121	18	4	3	4	3	BA DA DY
SST ^{3,4}	2	36	5	1	1	1	1	BA DA DY
NDF	409	351	407	373	397	406	333	BA DA DY
DNDF ³	373	326	376	345	360	364	311	... DA DY
Lignin	12	10	13	13	14	27	12	BA ...
N	28.9	27.1	25.8	33.6	27.9	27.1	31.3	BA DA DY
SN ³	5.6	8.5	6.5	16.8	12.4	10.1	14.0	BA ... DY
UN ³	1.1	1.2	1.4	1.1	1.4	1.6	1.1	... DA DY
NH ₃ -N	1.4	2.1	1.6	4.2	3.1	2.5	3.5	BA DA DY
Ash	103	90	103	90	103	105	111	BA ...
Lipid	39	41	31	37	37	41	37	BA DA DY
Parameter inputs								
Intake, kg/d	16.2	16.3	16.5	13.3	16.8	13.0	15.2	BA DA DY
kd_{DNDF}^3 , /d	1.25	1.56	1.65	1.41	1.24	1.10	1.64	... DY
kd_{DP}^3 , /d	1.97	2.08	1.87	2.12	2.17	1.53	2.14	... DY
kd_{DST}^3 , /d	3.00	2.71	3.00	3.00	3.00	3.00	3.00	... DY

 continued									
kp_{liq} , /d	3.72	3.89	3.89	4.51	4.13	2.86	2.26	BA	...	DY
kp_{OM} , /d	0.77	1.22	0.94	0.79	0.82	0.58	0.58	BA	...	DY
$k_{p,sp}$, /d	4.5	4.5	4.5	4.5	4.5	4.5	4.5	BA
p_{sf} ⁶	0.4	0.4	0.4	0.4	0.4	0.4	0.4	BA
G ⁶	0.04	0.04	0.04	0.04	0.04	0.04	0.04	...	DA	...
pH	6.14	6.16	6.15	6.60	6.10	6.20	6.10	BA	...	DY
$pH-min$	5.78	5.87	5.86	6.20	5.80	5.85	5.80	DY
Tf_{63} , h	9.5	8.5	8.5	0.0	6.0	5.0	6.0	DY
Tf_{60} , h	8.0	7.0	7.0	0.0	5.0	4.5	5.0	DY
V_{Ru} , L	64.5	53.8	57.7	54.6	64.0	67.8	72.9	BA	DA	DY
F_{cell}	0.53	0.48	0.53	0.49	0.46	0.48	0.48	BA	DA	DY

¹Abbreviations: SC = soluble carbohydrate, DST = insoluble, degradable starch, SST = soluble starch, DNDF = degradable NDF, SN = soluble N, UN = undegradable N, kd_{NDF} = degradation rate NDF, kd_{DP} = degradation rate protein, kd_{DST} = degradation rate starch, kp_{liq} = fractional liquid passage rate, kp_{OM} = fractional OM passage rate, $k_{p,sp}$ = fractional comminution rate of large particles, p_{sf} = particle size factor, G = partitioning factor of total carbohydrate fermentation in sugars, starch, and NDF fermentation, pH = mean pH, $pH-min$ = minimum pH, Tf_{63} = time interval pH <6.3, Tf_{60} = time interval pH <6.0, V_{Ru} = rumen non-DM, and F_{cell} = fraction cellulose in NDF.

²Diets 1 through 3, ryegrass herbage with one-third of the grass replaced by concentrates in diets 2 and 3; diets 4 through 7, ryegrass herbage differing in N fertilization rate and harvesting period.

³Estimated from nylon bag incubations.

⁴With the exception of diet 2, SST was assumed to be 20%.

⁵Assumed that 25% of soluble N was of non-aminogenic origin and immediately hydrolyzed to NH_3 ; diets contained no NH_3 .

⁶Not experimentally estimated.

Removal of urea and inclusion of soluble protein pools in the DA. Two adaptations in the DA3 created a description of the destination of protein and NH₃ inputs that is more comparable with that of the other models.

First, the DA lacks mathematical distinction between soluble and degradable rumen protein pools. Division of the single pool of available protein in separate pools of soluble and of insoluble, degradable protein with associated fractional passage rates in the DA3 improved correspondence with the other models. Because degraded protein constitutes an input to the soluble protein pool, the soluble protein pool was included between the available protein pool and the microbial pool in the original model (Equations [7] through [12] in Table A3). The same relationship of microbial protein uptake from the available protein pool in the original model was used for both protein flow from the insoluble, degradable to the soluble protein pool and for microbial protein uptake from the soluble protein pool in the DA3 (Equations [8] & [12] in Table A3). Accordingly, protein input was divided into soluble and degradable fractions (Table 1) for the corresponding protein pools (Equations [7] & [9] in Table A3).

Second, in the DA, a separate rumen urea pool is envisaged from which NH₃ flows to the rumen NH₃ pool by urea hydrolysis (Equations [1] & [2] in Table A3). Because the BA and DY apply instantaneous conversion of urea input to the rumen NH₃ pool, the urea pool was removed, and urea input was instantaneously supplied into the NH₃ pool (Equation [6] in Table A3) in the DA3.

Table 2. Sequential adaptations of the models of Baldwin *et al.* (1987; BA) and Danfær (1990; DA) with parameters, equations, and concepts of the model of Dijkstra *et al.* (1992; DY).

	Model		
Original model with experimental rumen volume	...	DA0	...
Original model with experimental fractional passage rates	BA1	DA1	DY1
Non-dietary inputs	BA2	DA2	...
Inclusion of soluble protein pool, removal of urea pool	...	DA3	...
Absorption equations	BA4	DA4	...
Degradation equations	BA5	DA5	...
Inclusion of undegradable protein and NDF pools	BA6
Molecular weights	BA7	DA7	...
Parameters of VFA production	BA8	DA8	...
Removal of large particle pool	BA9

Absorption of VFA and NH₃. In the BA and DA, absorption rates of VFA and NH₃ are assumed to be linear in their rumen concentrations. Dijkstra *et al.* (1992), however, implemented a dependency on rumen pH, rumen volume, and rumen concentration. With this adaptation, mathematically identical equations were introduced in the BA4 and DA4 (Table A4).

Degradation rates of NDF, starch, and protein. Mathematically identical equations for degradation rates of NDF, insoluble starch, and insoluble protein were impossible to create because the microbial elements involved were conceptually different. Therefore, degradation rate equations of the DY were incorporated in the BA and DA; cellulolytic (utilizing NDF) and amylolytic (utilizing easily fermentable carbohydrates) microbe pools in the DY (Equations [19] through [21] in Table A5) were replaced by pools of microbes bound to NDF, starch, and small particles in the BA5 (Equations [4 through [6] in Table A5) and the microbial CP pool in the DA5 (Equations [10] through [12] in Table A5). Applied units were made corresponding. In the BA5, the maximum rate of starch and protein degradation had to be adjusted by multiplication with 100 and 10, respectively, to obtain results that were comparable with the DY (Equations [4] & [6] in Table A5). The BA and DY contain pools of soluble carbohydrate and protein for collection of ingested soluble and degraded insoluble carbohydrates and protein, respectively. According to these model descriptions, microbes only utilize these solubilized carbohydrates and protein. The DA, however, formulated microbial utilization directly from carbohydrate pools for sugars, starch, NDF, and protein without intervening soluble carbohydrate and soluble protein pools. Therefore, simultaneously with adaptation of the degradation equations, a collection pool of soluble carbohydrates was inserted in the DA5 (Table A5), which created a description of substrate availability for microbial utilization that was comparable with the DY and BA. This pool receives flows of ingested soluble carbohydrates, degraded starch, and degraded NDF as inputs (Equation [15] in Table A5). Equations for microbial carbohydrate utilization in the DA0 were applied to this soluble carbohydrate collection pool (Equations [16] & [17] in Table A5). A soluble protein pool had already been included in the DA3 (previous adaptation).

Inclusion of undegradable protein and NDF pools in the BA. The BA assumes that only the lignin part of NDF is undegradable and that ingested protein is totally degradable. *In situ* data (Van Vuuren *et al.*, 1992, 1993) clearly indicate the larger undegradable NDF and protein fractions (Table 1) that were used as input in the DA and DY. The BA6 was altered to conform more to the DA and DY by inclusion of an undegradable protein pool, replacement of the lignin pool by an undegradable NDF pool, and application of *in situ* fractions as input in the BA6.

Molecular weights and glycerol fraction in dietary lipid. The models assume different molecular weights of substrates and glycerol fractions in dietary lipid. In addition, the BA assumes an increase in molecular weight with substrate solubilization. The effect of these differences on model behaviour was examined by applying values of the DY in the BA7 and DA7 (Table A6).

VFA production parameters. Each model assumes its own set of coefficients that determine which VFA are produced with fermentation of sugars, starch, hemi-cellulose, cellulose, and protein (Table A7). The influence on predicted production and molar percentages of acetate, propionate, and butyrate was examined by application of parameter values of the DY in the BA8 and DA8. The DY also considers valerate formation, which was omitted in the present study and not accounted for in the DY1 during model comparison. Additionally, coefficients of ATP yield in the BA were applied in the DA8 (Table A7).

Removal of large particle pool in the BA. Only the BA contains a description of particle dynamics, which creates a distribution of insoluble substrate over large and small particle pools. Microbial substrate utilization is restricted to small particles and solubilized substrate. Substrate within the large particle pool, which only becomes available after comminution, cannot leave the rumen (Baldwin *et al.*, 1987). The consequences were investigated of removal of this representation of particle dynamics by setting the particle partition factor of ingested DM to 1.0 in the BA9. As a result, no substrate entered the large particle pool, and, thus, rumen substrate was wholly available for microbial utilization, which corresponded to the formulation in the other models.

Remaining Differences in Microbial Mechanisms

Formulations of microbial synthesis were compared after all of the adaptations by consideration of simulated destinations of inputs of rumen carbohydrate and N. Because the BA does not contain intra-ruminal recycling, calculations were straightforward. In the DA and DY, however, the recycling mechanisms had to be taken into account. Therefore, flows were calculated that directly originated from rumen inputs of N and carbohydrates (excluding the N and carbohydrate inputs in rumen substrate pools that originate from conversions between rumen pools instead of ingestion). These flows were used in the calculation of the destination of dietary rumen inputs of N and carbohydrates in terms of microbial synthesis, incorporation in microbial mass, fermentation into VFA, and passage from the rumen.

The influence of specific formulations on model behaviour was examined by comparison of sequential model versions from BA1 to BA9 and DA0 to DA8. Results are given for every adaptation and are expressed as the percentage of change in model prediction averaged over the seven diets. Remaining differences between the BA9, DA8, and DY1 are given for each diet.

Table 3. Mean percentage change in behaviour of the Danfær (1990) model (DA) with sequential adaptations.

	Version DA model							Mean deviation DA8 vs. DY1 ¹
	1 vs. 0	2 vs. 1	3 vs. 2	4 vs. 3	5 vs. 4	7 vs. 5	8 vs.7	
	(%)							(%)
FSC ²	-5	0	1	0	-19	0	0	36
FST	-1	0	2	0	-25	0	0	-15
FNDF	5	-2	10	0	21	0	0	15
FP	13	1	-45	-13	9	-3	39	-46
FOM	2	-1	-3	-1	1	0	2	6
DST	-1	0	-18	-1	-1	0	0	12
DNDF	4	0	1	0	8	0	0	-2
DP	7	1	-36	0	-1	0	0	-2
DOM	5	1	-12	0	5	0	0	-1
PFSC	0	2	88
PTSC	252	3	2	0	946	-4	2	374
PMST	15	5	8	9	-77	36	-32	-94
PFST	42	3	-16	0	154	0	1	-71
PTST	21	4	-7	9	-64	11	-18	-91
PNDF	-11	1	-4	0	-27	0	-1	14
PMP ³	-1	-1	0	-1	0	1	1	-8
PFP	-22	1	149	3	-5	-3	-1	72
PTP	-8	-1	36	1	-2	-1	0	67
PMOM	4	1	-10	2	-30	1	-1	-39
PFOM	-5	0	21	5	32	-2	0	67
PTOM	-1	1	4	4	4	-1	-1	9
NH ₃	-8	-15	0	111	0	0	0	262
VFA								
Concentration	2	-1	-12	44	1	2	2	8
Production	2	-1	-12	-1	1	1	0	6
Acetate, mol/100 mol	0	0	10	2	3	0	12	7
Propionate, mol/100 mol	-4	7	-26	-4	-10	0	-43	-32
Butyrate, mol/100 mol	-2	1	10	-7	1	3	6	1

¹Model of Dijkstra *et al.* (1992).

²Abbreviations: FSC = fermented soluble carbohydrates, FST = fermented starch, FNDF = fermented NDF, FP = fermented protein, FOM = fermented OM, DST = degraded insoluble starch, DNDF = degraded NDF, DP = degraded insoluble protein, DOM = degraded insoluble OM, PFSC = passage of feed soluble carbohydrates (inclusive solubilized NDF, exclusive solubilized starch), PTSC = total passage of soluble carbohydrates, PMST = passage of microbial starch, PFST = passage of feed starch (inclusive solubilized starch), PTST = total passage of starch, PNDF = passage of NDF (exclusive solubilized NDF), PMP = passage of microbial protein, PFP = passage of feed protein, PTP = total passage of protein, PMOM = passage of microbial OM, PFOM = passage of feed OM, and PTOM = total passage of rumen OM excluding VFA.

³Microbial N was assumed to consist of 80% protein N and 20% NPN.

Table 4. Mean percentage change in behaviour of the model of Baldwin *et al.* (1987; BA) with sequential adaptations.

	Version BA model							Mean deviation DA9 vs. DY1 ¹
	2 vs. 1	4 vs. 2	5 vs. 4	6 vs. 5	7 vs. 6	8 vs. 7	9 vs. 8	
	(%)							(%)
FSC ²	1	-2	0	0	-5	0	-3	40
FST	0	-4	20	0	-10	1	-3	-19
FNDF	0	-1	0	-5	-11	0	-2	9
FP	-1	-4	13	-4	-13	1	-2	15
FOM	0	-2	4	-4	-10	1	-2	17
DST	0	-2	32	0	0	0	1	-7
DNDF	0	1	0	-5	0	0	1	-10
DP	0	-3	16	-6	0	1	2	-7
DOM	0	0	5	-5	0	0	1	-9
PFSC	138	-28	-8	10	-8	5	-15	-97
PTSC	5	-17	-8	6	-11	5	-14	-92
PMST	-2	6	3	-4	2	-1	12	106
PFST	1	5	-58	3	0	-1	-5	-42
PTST	-2	6	-8	-4	2	-1	11	79
PNDF	2	-2	0	25	0	0	-4	68
PMP ³	-2	6	3	-4	2	-1	12	13
PFP	1	10	-38	30	-1	-3	-6	-30
PTP	-1	7	-8	2	1	-1	8	-1
PMOM	-2	6	3	-4	2	-1	12	-6
PFOM	0	4	-11	19	0	0	-6	-8
PTOM	0	5	-4	6	1	-1	3	-7
NH ₃	-34	153	23	-4	9	3	-17	112
VFA								
Concentration	0	91	4	-3	2	-17	-3	22
Production	0	-2	4	-4	2	-17	-3	19
Acetate, mol/100 mol	0	4	-1	0	0	14	0	1
Propionate, mol/100 mol	0	-6	2	0	1	-29	0	1
Butyrate, mol/100 mol	0	-8	1	0	0	-18	0	-5

¹Model of Dijkstra *et al.* (1992).

²Abbreviations: FSC = fermented soluble carbohydrates, FST = fermented starch, FNDF = fermented NDF, FP = fermented protein, FOM = fermented OM, DST = degraded insoluble starch, DNDF = degraded NDF, DP = degraded insoluble protein, DOM = degraded insoluble OM, PFSC = passage of feed soluble carbohydrates (inclusive solubilized NDF, exclusive solubilized starch), PTSC = total passage of soluble carbohydrates, PMST = passage of microbial starch, PFST = passage of feed starch (inclusive solubilized starch), PTST = total passage of starch, PNDF = passage of NDF (exclusive solubilized NDF), PMP = passage of microbial protein, PFP = passage of feed protein, PTP = total passage of protein, PMOM = passage of microbial OM, PFOM = passage of feed OM, and PTOM = total passage of rumen OM excluding VFA.

Results

Effect of Adaptations on Model Behaviour

Experimental passage rates in the DA. Substitution of the fractional passage rates in the DA0 with experimental values (DA1) altered the simulated destination of sugars, starch, NDF, and protein (DA1 vs. DA0, Table 3). The destination of feed protein changed as degradation and fermentation increased and passage decreased. Because microbial protein passage was unaltered, total protein passage also decreased. Mean percentages of change for passage of soluble carbohydrate and starch were especially large because of their small absolute amounts in the DA0.

Urea transfer to the rumen and saliva input. Application of equations of the DY for salivary protein input and urea absorption across the rumen wall had a profound effect on non-dietary protein and urea input in the BA2 and DA2 (data not shown). Because the DA0 made no allowance for salivary protein, total protein supply was slightly increased, but non-dietary NH₃ input was reduced by 66%. Adaptation of the BA reduced non-dietary NH₃ and protein input 56 and 43%, respectively. Although total protein input did not change much (high N contents of diets), the reduction of non-dietary NH₃ input reduced rumen NH₃ concentrations in both models (DA2 vs. DA1, Table 3; BA2 vs. BA1, Table 4). Apart from the NH₃ concentration, the behaviour of the models was not severely altered (the large increase in soluble carbohydrates passage in the BA2 remained small absolute quantities).

Removal of urea and inclusion of soluble protein pools in the DA. Removal of urea and inclusion of a soluble protein pool in the DA3 did not influence NH₃ concentration, and availability of NH₃ thus remained unchanged. Subdivision of the protein pool into soluble and insoluble fractions reduced protein availability for microbial utilization, as illustrated by a large reduction in degraded and fermented protein (DA3 vs. DA2, Table 4). Because passage of microbial protein remained unaltered, the increased passage of total protein must be attributed to a larger amount of feed protein escaping the rumen.

Absorption of VFA and NH₃. Introduction of absorption equations from the DY increased the VFA and NH₃ concentrations in the DA4 (DA4 vs. DA3, Table 3) and BA4 (BA4 vs. BA2, Table 4), which indicates much lower absorption rates than in the original model formulations. Because the absorption rate equations of Dijkstra *et al.* (1992) are nonlinear, the relative alterations in absorption rate differed for individual VFA types, causing a change in simulated VFA patterns (Tables 3 and 4). Theoretically, VFA concentration is only a model output and therefore has no influence on the simulated rumen function. However, NH₃ does influence the rumen function as a substrate for microbial protein synthesis. Increased NH₃

concentration was accompanied by increased passage of microbial, feed, and total OM; a decreased fermentation of OM in the BA4 (BA4 vs. BA2, Table 4); and particularly a decrease in protein fermentation rate in the DA4 (DA4 vs. DA3, Table 3).

Degradation rates of NDF, starch, and protein. Adoption of degradation rate equations of the DY in the BA and DA resulted in more comparable amounts of degraded substrate over the seven diets (average deviation of starch, NDF, and protein degradation from the DY1 changed from 13, -9, and -1% in the DA4 to 12, -2, and -2% in the DA5, and from -30, -7, and -17% in the BA4 to -8, -6, and -3% in the BA5, respectively). The most pronounced changes were an increase in NDF degradation in the DA5 (DA5 vs. DA4, Table 3) and an increase in degradation of starch and protein in the BA5 (BA5 vs. BA4, Table 4). Unaltered degradation of NDF in the BA5 and protein in the DA5 were already comparable with the DY prior to adaptation. Despite increased degradation and unaltered fermentation of OM, the simultaneous insertion of a collection pool for soluble carbohydrates in the DA5 caused a severe decrease in the simulated utilization of feed OM and passage of microbial OM (microbial protein synthesis was unaltered, however).

Inclusion of undegradable protein and NDF pools in the BA. Application of *in situ* undegradable NDF and undegradable protein as model input in BA6 influenced model behaviour by a reduced rumen availability of NDF and protein. As a consequence, substrate degradation, substrate fermentation, and microbial growth were lowered. Thereby, passage of feed OM (especially NDF and protein) increased (BA6 vs. BA5, Table 4).

Molecular weights and glycerol fractions in dietary lipid. Because almost all molecular weights in the DA and DY were identical, this adaptation had only minor effects on behaviour of the DA (DA7 vs. DA5, Table 3), but it reduced fermentation rates in the BA (BA7 vs. BA6, Table 4). Although passage of starch in the DA7 and passage of soluble carbohydrate in the BA7 were changed, the absolute quantities remained small.

An interesting characteristic of the BA6 was the generation of OM by simulation of higher amounts of OM that fermented or passed rather than ingested. This characteristic was caused by the assumption of higher molecular weights in the solubilized than in the undegraded state of substrates. Application of equal molecular weights for both states in the BA7 resulted in a more comparable OM flow for the BA7 and DY1 (passed and fermented OM : ingested OM ratio changed with -0.06 to 0.99). The DA7 displayed almost no change in model behaviour (small absolute increment in the microbial non-protein fraction), and amounts of ingested OM were almost equal to amounts of fermented and passed OM (ratio changed with -0.01 to 1.01). The DY1 showed a loss of OM (ratio of 0.95).

VFA production parameters. With application of identical parameters for formation of acetate, propionate, and butyrate, VFA production remained almost unaltered in the DA8 (DA8 vs. DA7, Table 3), in contrast to a strong decrease in the BA8 (BA8 vs. BA7, Table 4). Additionally, VFA patterns were severely altered in both models by increased and decreased molar percentages of acetate and propionate, respectively. Simulated VFA patterns were similar between the BA8 and DY1, but the DA8 simulated different VFA patterns because fermented amounts of individual substrate types differed.

Removal of large particle pool in the BA. More substrate utilization and microbial growth, accompanied by a slight decrease in OM fermentation (BA9 vs. BA8, Table 4), was provided by removal of the large particle pool in the BA. Changes in simulated flows were relatively small (Figure 1B), in contrast to large alterations of rumen pool sizes (Figure 1A). The mean change in microbial OM passage, total OM passage, and fermented OM was 12, 3, and -2%, respectively, compared with a change of -35 and -48% for microbial and total OM pool size, respectively.

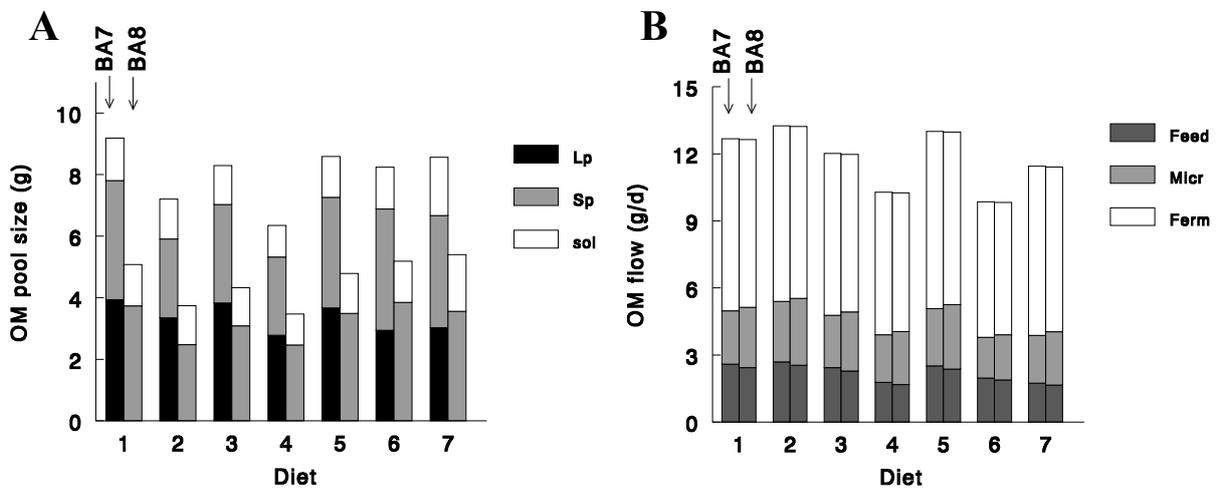


Figure 1. Effect of removal of particle dynamics mechanism on (A) pool sizes of large particles (Lp), small particles (Sp), and soluble OM (sol) and on (B) passage of microbial OM (Micr), non-microbial OM (Feed), and fermented OM (Ferm). BA7 and BA8 are adapted versions from the model of Baldwin et al. (1987; BA), with and without the particle dynamics mechanism, respectively.

Remaining Differences with Equal Microbial Environments

After all adaptations, the models simulated similar amounts of degraded NDF, starch, and protein (Figure 2A), but, in particular, the amount of degraded NDF in the BA9 did not attain that of the DA8 and DY1.

Microbial substrate utilization was divided into substrate fermentation and incorporation (microbial synthesis). The BA9 and DA8 simulated more fermented inputs of rumen carbohydrates than the DY1; only the BA9 differed consistently (Table 5; Figure 2D). An increased fraction of the inputs of rumen carbohydrates passed from the rumen (Table 5, Figure 2B), in the order DY1, BA9, and DA8. Furthermore, incorporated inputs of rumen carbohydrates (Table 5) and passage of synthesized non-protein microbial matter decreased (Figure 2C). Because carbohydrates were additionally used for protein synthesis, less non-protein microbial matter was synthesized than carbohydrates were incorporated.

A larger fraction of input of rumen protein was utilized and a smaller fraction passed from the rumen, in the order DA8, DY1, and BA9 (Table 6; Figure 2B). Subdivision of the total utilization of inputs of rumen protein into fermentation and incorporation showed this same order in increased daily fermentation (Table 6; Figure 2D) and decreased incorporation (Table 6). A contradictory order in protein synthesis from NH_3 (Table 6) compensated differences in incorporation and fermentation, resulting in more comparable daily microbial CP synthesis from inputs of rumen N (Table 6; Figure 2C).

Because the same VFA production parameters, fractional liquid passage rates, and absorption rate equations were applied in the three models, simulated OM fermentation (Table 6; Figure 2D) corresponded to VFA concentrations (Figure 3A). Additionally, simulated NH_3 concentrations reflected differences in the balance between protein fermentation and microbial CP synthesis from NH_3 in the BA9 and DY1 (Table 6; Figure 3B), but not in the DA8.

Discussion

Extra-microbial Processes

Because of sensitivity of the BA and DA to most of the adaptations, selection of a specific formulation strongly influences model performance. Those features for which the models demonstrated sensitivity had large implications for the simulated amounts and profiles of nutrients absorbed and passed to the duodenum. Hence, in model evaluation, the discrimination between alternatives to formulation is not trivial.

Fluid and particle passage parameters in the DA and elimination of the physical differentiation in large and small particle pools of the rumen in the BA had important consequences for simulation results. This result is in agreement with published results (Baldwin *et al.*, 1987; Dijkstra, 1993; Neal *et al.*, 1992) of sensitivity analysis for rumen

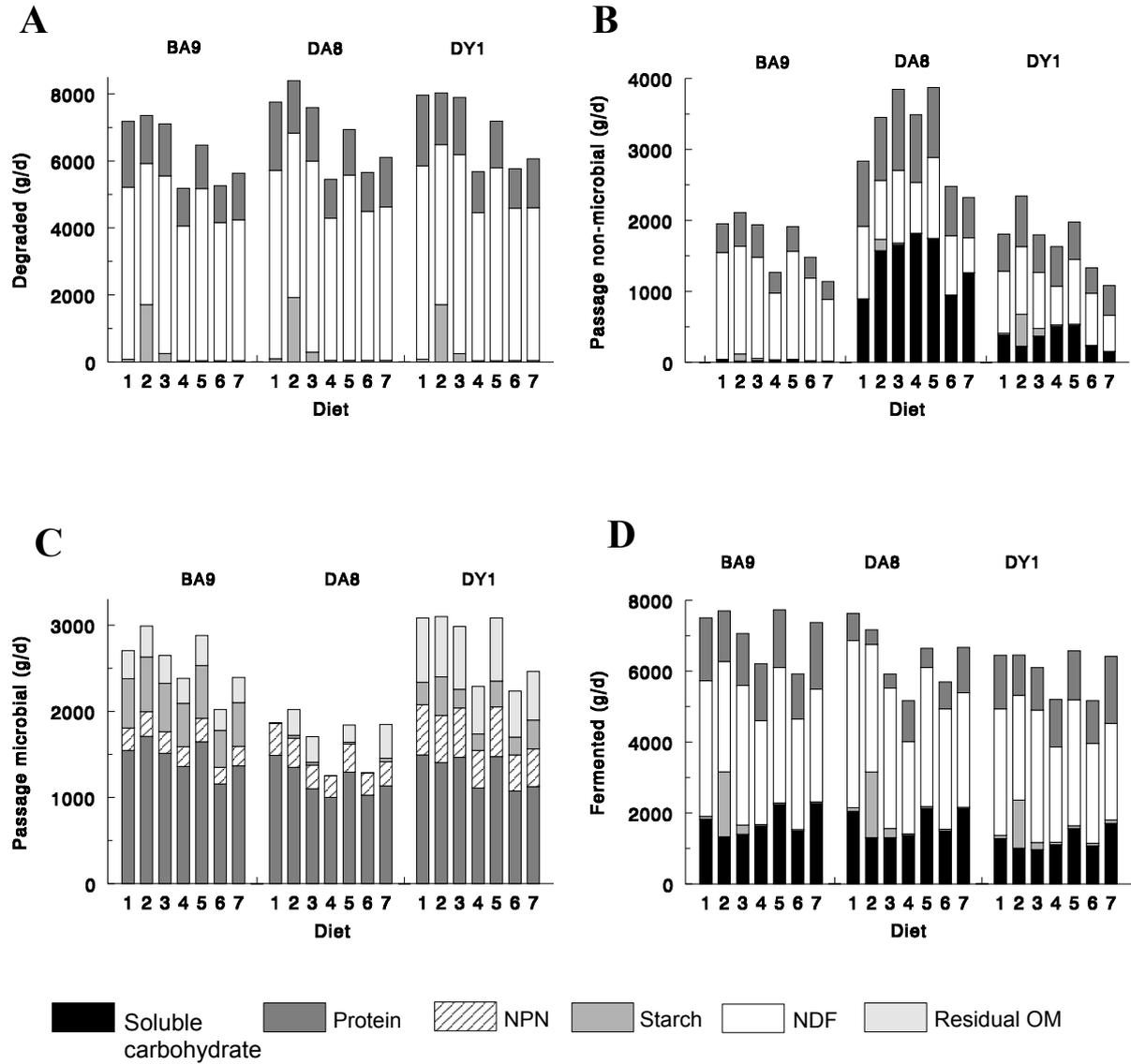


Figure 2. Simulated (A) degradation of starch, NDF, and protein; (B) fermentation of soluble carbohydrates, starch, NDF and protein; (C) passage of soluble carbohydrates (including solubilized NDF), starch (including soluble starch), NDF, and protein of non-microbial origin; and (D) passage of microbial protein, non-protein, starch, and residual OM, in the DY1, DA8 and BA9 models (model of Dijkstra et al. (1992), Danfær (1990), and Baldwin et al. (1987), respectively; numbers are adaptations). Microbial N in the DA8 model was assumed to consist of 80% protein N and 20% NPN.

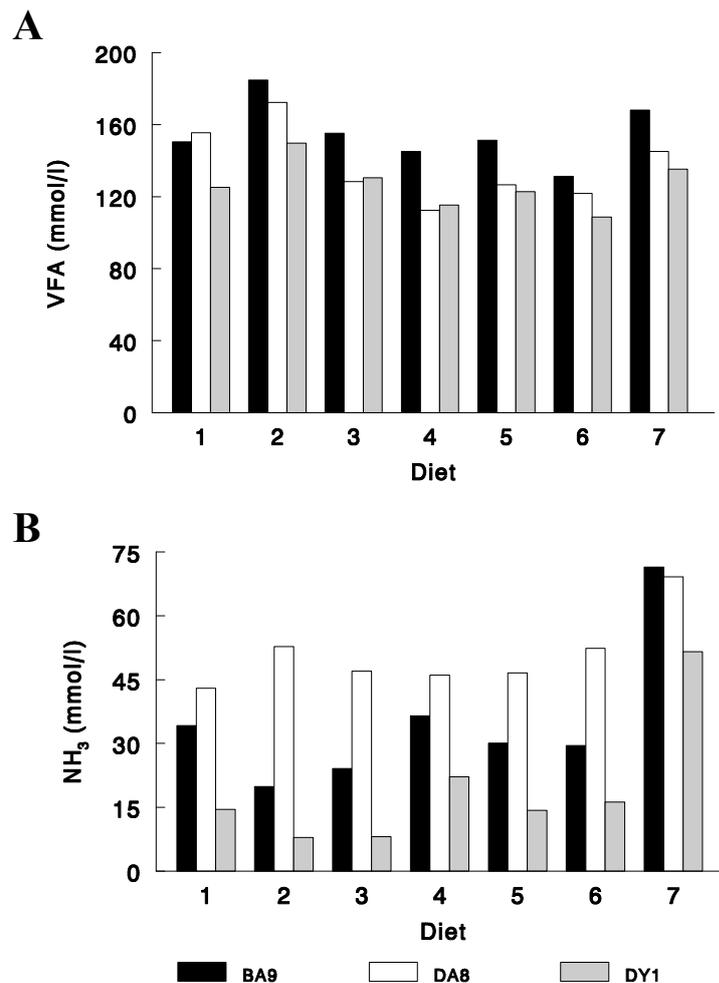


Figure 3. Simulated concentrations of (A) VFA and (B) NH₃ in the DY1, DA8, and BA9 models (model of Dijkstra *et al.* (1992) and models of Danfær (1990) and Baldwin *et al.* (1987), respectively; numbers are adaptations).

models, and these concepts are generally recognized as important in the understanding of rumen function. Not surprisingly, therefore, the distinction between soluble and degradable substrate pools in the DA also appeared to be important. But, as a consequence of this sensitivity, proper parameter inputs for passage rates that are quantified with methods similar to the ones used to estimate the parameter values on which the models were calibrated are a prerequisite for accurate predictions of flow. Much research is concerned with the characterization of particle dynamics in varying dietary situations (Ellis *et al.*, 1984; Faichney, 1986; Owens & Goetsch, 1986), but current mechanistic rumen models are limited in describing these aspects of rumen function. Above all, these models are explanatory for chemical aspects of microbial activity. Removal of the mechanism of particle dynamics from the BA8 resulted in relatively small changes in simulated flows of OM, however, compared with a severe decrease in rumen pool sizes of OM (Figure 1). Pool sizes are thus strongly dependent on the presence of a delay in substrate availability. Therefore, with other model

goals, such as prediction of intakes, rumen pool sizes have to be predicted accurately (Mertens, 1987), and some description of a delay or time lag in degradation rates, dynamics of distinct particle fractions, or some other heterogeneity must be embodied in rumen models.

The significance of N recycling to the rumen is often emphasized (Beever, 1984), but, in the present study, relatively large alterations to the recycling rate did not affect simulated rumen function. This result can be explained by the high N content in the diets, which reduced the importance of N recycling and prevented large changes in microbial growth rate. Although rumen NH_3 concentration was influenced by adaptation of N recycling, the additional effects were small. Nevertheless, adopted N recycling rates probably have much larger implications for model predictions on low N diets. Correct representation of N recycling to the rumen is also important when whole animal N metabolism is considered (Beever, 1984).

Although equations for VFA absorption and parameters for VFA production only concern model outputs and, thus, do not influence simulated rumen function, correct values are important for accurate prediction of nutrient flows (Bergman, 1990). If assumptions on passage and absorption rates are incorrect, simulated VFA and NH_3 concentrations cannot be used as an indication of *in vivo* production or utilization rates. For example, Dijkstra (1993) demonstrated pronounced differences in VFA absorption characteristics *in vivo* that were dependent on VFA concentration, rumen pH, and rumen liquid volume. These relationships clearly indicate that the VFA patterns produced need not correspond to VFA patterns in the rumen. Predicted NH_3 concentrations corresponded to the extent of protein fermentation in the BA and DY. The DA differs from the BA and DY by a separate description of AA degradation to NH_3 from VFA formation with protein fermentation. Moreover, an explicit input to the NH_3 pool via protein fermentation is absent or balanced with other processes that consume NH_3 , and, therefore, only the energetic part of VFA production has been explicitly addressed. Consequently, N dynamics in the DA were not comparable with those in the BA and DY in simulating higher NH_3 concentration (Figure 3B) and lower protein fermentation (Figure 2D).

Microbial Mechanism

The rumen models conceptually contain very different microbial pools, but, after adaptations, the gained comparability in model response on substrate degradation with input variation was satisfactory. Although the NDF degradation remained lower in the BA9, further attempts to obtain values that were more comparable with those of the DY1 did not influence simulation results to such an extent that conclusions of model differences should be reconsidered. Conversely, many differences between models would become more pronounced. In addition to this comparable substrate degradation, the BA9, DA8, and DY1 were identical in the distinguished rumen substrate pools, passage rates, and absorption equations, which allowed the assumption that the formulation of substrate availability in the

microbial metabolic environment was similar for all three models. Thus, after all adaptations, remaining differences in simulated pool sizes and flows must be explained from the mathematical formulation pertaining to the unadapted microbial mechanisms only. Large differences remained in simulated rates of microbial fermentation, incorporation, and synthesis (Tables 5 and 6). It is interesting to relate these differences to specific features of the alternative microbial mechanisms.

Microbial recycling. An option with rumen modelling is to account for recycling aspects within the rumen (Beever, 1984; Williams & Coleman, 1991). In the DY, a substantial fraction of microbial matter is recycled with engulfment of bacterial mass by protozoa and death and lysis of protozoa. Also, the DA contains numerous recycling loops. As a consequence, simulated total daily microbial synthesis exceeds the microbial outflow and the microbial synthesis directly from inputs of rumen substrates (Tables 5 and 6). These recycling processes contribute to simulated differences in the microbial mechanism and consequently nutrient flows with rumen absorption and passage to the duodenum (Figure 2B & C; Figure 3). In an updated version of the DY, the representation of protozoal function (recycling) was improved, and Dijkstra (1993) demonstrated a strong influence of intake and roughage: concentrate ratios on microbial OM turnover and microbial growth efficiency. Therefore, more contrasting diets would probably add to the distinctions in model responses established in this comparison study.

Substrate-specific microbial growth. Formulation of substrate-specific growth is another important feature that is closely related to the basal microbial elements. There is experimental evidence for reduced microbial utilization of NDF as the proportions of easily fermentable carbohydrates increase in the diet, because of a shift towards the utilization of easily fermentable carbohydrates (De Visser, 1993). This effect is represented in rumen models by a separation of this cellulolytic and amylolytic microbial activity. Although such a substrate specificity was implemented differently in the models, input variation gave similar model responses of substrate degradation before and after the inclusion of the equations of Dijkstra *et al.* (1992) for substrate degradation (data not shown). Larger distinctions between the alternative formulations of substrate specificity will probably come forward only with diets that differ more from one another. Furthermore, substantial evidence exists for reduced cellulolytic microbial activity with low rumen pH (De Visser, 1993; Tamminga & Van Vuuren, 1988). This concept was implemented in the DY and BA. Another reason for qualitatively similar rates of simulated NDF degradation, despite the varying approaches in modelling, is probably the limited range of rumen pH <6.3 (Dijkstra *et al.*, 1992) or 6.2 (Argyle & Baldwin, 1988) of the seven diets (Table 1). In conclusion, differences in simulated substrate-specific microbial growth in the present study did not arise from differences in substrate degradation but were mainly caused by the distinct mathematical formulation of the

utilization of soluble substrates and the recycling mechanism. Implementation of the equations of Dijkstra *et al.* (1992) for substrate degradation and inclusion of a soluble carbohydrates collection pool for degraded carbohydrates in the DA eliminated Danfær's original formulation of substrate specificity (Danfær, 1990). However, the formulation of the microbial mechanism was not essentially altered by these adaptations, because the equations for substrate specificity were implemented non-mechanistically and independently of the equations for microbial substrate utilization. Mathematical equations for carbohydrate utilization remained the same, allowing for a comparison with the other models. However, variables that represent carbohydrate availability were altered from total rumen pool sizes (Equations [7] through [12] in Table A5) to pool sizes of available substrate in soluble form (Equations [13] through [17] in Table A5). This alteration reduced carbohydrate incorporation in microbial mass. Additionally, elimination of the original substrate-specific utilization with insertion of a collection pool of soluble carbohydrate changed the partitioning of total utilization over utilization of the individual carbohydrate types (DA5 vs. DA4, Table 4). This shift caused less ATP yield (ATP production parameters differ for individual carbohydrate types) and, consequently, reduced microbial non-protein passage and total carbohydrate utilization (DA5 vs. DA4; Table 4). Therefore, the established differences in total carbohydrate utilization between the DA8 and the BA9 and DY1 (Tables 3 and 4) can largely be ascribed to the inclusion of a soluble carbohydrate pool. In conclusion, although the BA9, DA8, and DY1 revealed substantial differences in simulated microbial substrate utilization (Figure 2B, 2C & 2D; Tables 5 & 6) with roughly similar substrate availability in rumen fluid; the DA8 in particular was used uncalibrated. The results clearly illustrate, however, the large differences in conceptual approach and their consequences for simulation of rumen function.

Substrate fermentation. Separate mathematical equations described total carbohydrate and protein fermentation in the BA and DA. Dijkstra *et al.* (1992), however, describe a number of fermentation processes in the rumen (growth on rumen substrates, predation, protozoal growth on engulfed bacteria, starch storage, and maintenance). Despite distinct microbial mechanisms, differences in VFA and NH_3 concentrations are highly systematic and almost constant (Figure 3). Apparently, resemblance is remarkably close in simulated VFA and NH_3 concentrations by the BA9 and DY1, which indicates that these two models respond qualitatively in a very similar way to input variation.

Microbial composition, maintenance, incorporation, and yield. The anabolic process of microbial growth yield on available ATP (Y_{ATP}) is closely related to the catabolic process of substrate fermentation; ATP utilization and production, respectively, are generally considered as intermediary. Growth rate (Russell & Strobel, 1993; Russell & Wallace, 1988) and microbial composition have a large influence on Y_{ATP} (Russell & Strobel, 1993; Russell &

Table 5. Simulated fate of rumen inputs of carbohydrates (ICH) in final model¹ versions.

Model	Diet				Mean deviation from DY1 model (%)
	1	2	3	4 (g/d)	
BA9	1548	1636	1481	977	13
DA8	1918	2563	2705	2539	100
DY1	1284	1632	1266	1074	
				ICH passed ²	
BA9	1934	2173	1908	1687	-35
DA8	432	768	762	715	-75
DY1	3025	3195	2853	2372	
				ICH incorporated ^{2,3}	
BA8	5729	6273	7056	4600	23
DA8	6861	6751	5520	4010	22
DY1	4902	5255	4868	3818	
				ICH fermented ²	
				6103	
				4649	
				6104	
				4938	
				5144	
				3922	
				5492	
				5390	
				4468	

¹ BA9 = Model of Baldwin et al. (1987) containing all of the adaptations, DA8 = model of Danfær (1990) containing all of the adaptations, and DY1 = original model of Dijkstra et al. (1992).

² Carbohydrate originating from intra-ruminal recycling was excluded.

³ ICH incorporated = (ICH - ICH fermented - ICH passed).

Wallace, 1988), and, additionally, microbial composition is a function of growth rate (Mulder, 1988). The Y_{ATP} can be significantly influenced by microbial composition because the ATP expense for synthesis of individual constituents of microbial mass differs (Hespell & Bryant, 1979). The variable polysaccharide content in microbial matter is particularly important (Czerkawski, 1986; Russell & Strobel, 1993), and such a concept was included in the DY. The BA and DA assume a constant composition of total microbial mass and non-CP microbial mass, respectively, and thus did not account for the most significant variation in microbial composition. Furthermore, the BA, DA, and DY partition microbial constituents, other than polysaccharide (starch), differently in cell wall, lipid, nucleic acids, and true protein. Accordingly, different flows to the duodenum were simulated (Figure 2C).

However, microbial composition is not the most important factor in explaining variation in experimental Y_{ATP} . Russell and Wallace (1988) state that the main impact from growth rate on Y_{ATP} is not via changes in cell composition, but rather is through its effect on the proportion of energy that is channelled to maintenance requirements and spilling. This aspect of energy requirements for maintenance and spilling was implemented differently in each model. In the BA, microbial maintenance (as total non-growth energy utilization) is assumed to increase linearly from 20 to 40 mol of ATP/kg of microbial OM per d with a decrease of rumen pH from 6.2 to 5.4. Because of the small range of pH values <6.2 in the present study, simulated maintenance requirements were almost constant. Danfær (1990) did not explicitly consider a maintenance requirement. In the DY, microbial growth is not determined by ATP production with substrate fermentation, as in the BA and DA, but is described as a function of amylolytic or cellulolytic hexose in rumen fluid. In contrast to the BA and DA, Dijkstra *et al.* (1992) implemented an increasing maintenance (non-growth) requirement of hexose when the supply of rumen NH_3 and soluble protein is relatively limiting. Instead of this variable maintenance requirement in the DY, the BA and DA use the concept of a decreasing Y_{ATP} with decreasing rumen availability of AA and NH_3 . Notwithstanding these differences in mathematical description, the variable Y_{ATP} and the constant (in this study) or no maintenance requirement of ATP in the BA and DA, respectively, are functionally similar to the variable maintenance requirement of hexose in the DY.

The BA and DY assume a constant stoichiometry for microbial biosynthesis, but the DA differs with a Y_{ATP} equation for synthesis of microbial protein instead of total mass and separate interrelated pools for other microbial constituents. In addition to use of ATP or hexose as energy source, substrate incorporation is required for microbial biosynthesis. All three models describe microbial biosynthesis by separate equations for synthesis from AA or NH_3 as the N source, a generally made distinction (Hespell & Bryant, 1979; Stouthamer, 1973; Wallace & Cotta, 1988). Only the DY describes a dependency on hexose availability in addition to AA and NH_3 availability; an increase in hexose availability provides a differential increase of microbial utilization of AA and NH_3 . However, the formulation of these two

distinct growth types was different between models, and simulated requirements of incorporated substrate for microbial synthesis deviated greatly (carbohydrates, Table 5; AA and NH₃, Table 6). These differences were caused by distinct parameterizations for microbial composition and hexose, AA, and NH₃ requirements for biosynthesis of microbial mass. Moreover, only the BA and DA assumed incorporation of dietary fatty acids, and only the DY considered variable polysaccharide storage in microbial mass. The models also differed in simulated OM losses with microbial biosynthesis and rumen recycling processes.

After identification of these conceptual approaches on microbial composition and requirements for growth and maintenance, the precise impact on simulated microbial yield and efficiency remains to be established. Truly implemented Y_{ATP} , instead of apparent values derived from duodenal flows of microbial mass, can be estimated from the fractions of substrate inputs that were used for microbial synthesis and fermentation (Tables 5 and 6). Although the BA9 simulated more substrate fermentation than the DY1, this difference is broadly compensated by the lower amount of ATP assumed to be generated with hexose fermentation (4.0 and 4.5 mol ATP/mol of fermented hexose in the BA and DY, respectively). Because the DY1 simulated a higher passage of microbial mass, the Y_{ATP} in the DY1 exceeded that in the BA9. The DA8 simulated a much lower passage of microbial mass to the duodenum than the BA9 and DY1 (Figures 2C & D; Tables 3 & 4), caused by the decrease of non-CP microbial mass with adaptation from the DA4 to the DA5. But, in the DA0, the simulated passage of microbial protein as well as non-protein corresponded more to those of the BA and DY (data not shown). Because of recycling and the higher ATP yields with carbohydrate fermentation (Table A7), Y_{ATP} in the DA0 also exceeded that in the BA.

Measures of the apparent efficiency of microbial growth confirm this order. For example, the DY1 simulated a higher passage of microbial OM per truly digested OM than the BA9 (Table 4). Another measure of apparent microbial efficiency, passage of microbial protein N per truly digested OM, shows the same order (Figures 2B & 2C). Although the passage of microbial protein was highest in the BA9, the passage of total microbial N was >10% higher in the DY1 because a higher microbial NPN content was assumed (Figure 2C). The DA8 showed the lowest apparent microbial efficiencies for reasons discussed previously. The original models simulated increasing apparent efficiencies in the order BA, DA and DY (Dijkstra *et al.*, 1992) (data not shown).

For microbial composition, maintenance requirement, substrate incorporation, and microbial yield, the differences between the BA, DA, and DY have large consequences for the simulated microbial role in rumen function. Further insight and an improved mathematical description of *in vivo* microbial function is of interest not only to evaluate predicted model outputs, but also to improve the definition of the microbial concepts applied in such models.

Conclusions

Generally, the precise background for different mathematical formulations is difficult to identify. Modelling goals, descriptions, applied concepts, mathematical equations, and experimental data strongly influence the eventual form of the model (Gill *et al.*, 1989; Thornley & France, 1984). Systematic differences between models can therefore be introduced. In model construction, unknown system parameters are optimized to calibrate the model for a selected reference experiment. Consequently, alternative rumen models necessarily produce approximately similar model outputs on which they were calibrated, but systematic differences in formulation of the underlying mechanism are not apparent from these outputs.

In the present study, these systematic differences were identified by substitution of specific mathematical equations or parameters and derivation of the consequences for model behaviour. However, the effect of a certain adaptation would not have been the same with a different order in sequential adaptation. Conclusions on adapted formulations and the microbial mechanisms can therefore only be extrapolated to the original models qualitatively. A wider range in intakes and in roughage: concentrate ratios could have elucidated a greater differentiation in model characteristics than was identified in the present study. However, results over the seven diets were consistent (data for individual diets not shown), which indicates that the conclusions are valid for varying dietary situations, although this finding has yet to be confirmed. An improved insight was gained on the contribution of microbial functions to overall model behaviour and the explanation of model differences for passed and absorbed nutrient flows. Although mechanistic rumen models can be equally structured, have similar concepts applied, and have similar outputs described, the mathematical description of individual processes in the mechanism is different. Further experimental identification and discrimination between the alternative models in mathematical description of these processes are necessary to improve predictions of nutrient flows.

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References

- Argyle, J.L. & Baldwin, R.L., 1988. Modeling of rumen water kinetics and effects of rumen pH changes. *Journal of Dairy Science* 71, 1178-1188.
- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54, 107-131.
- Beever, D.E., 1984. Some problems of representing nitrogen metabolism in mathematical models of rumen function. In: R.L. Baldwin & A.C. Bywater (Eds.) *Modeling Ruminant Digestion and Metabolism*. Proceedings 2nd International Workshop. University of California, Davis, United States of America, pp. 54-58
- Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70, 567-590.
- Czerkawski, J.W., 1986. *An Introduction to Rumen Studies*. Pergamon Press, Oxford, United Kingdom.
- Danfær, A., 1990. *A dynamic model of nutrient digestion and metabolism in lactating dairy cows*. PhD Thesis, Report 671, National Institute of Animal Science, Foulum, Denmark.
- De Visser, H., 1993. *Influence of carbohydrates on feed intake, rumen fermentation and milk performance in high-yielding dairy cows*. PhD Thesis, Agricultural University Wageningen, Wageningen, The Netherlands.
- Dijkstra, J., Neal, H.D.StC., Beever, D.E. & France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *Journal of Nutrition* 122, 2239-2256.
- Dijkstra, J., 1993. *Mathematical modelling and integration of rumen fermentation processes*. PhD Thesis, Agricultural University of Wageningen, Wageningen, The Netherlands.
- Ellis, W.C., Matis, J.H., Pond, K.R. & Mahlooji, M., 1984. Physical and chemical digestion of forage fragments with emphasis on stochastic heterogeneous rate models. In: R.L. Baldwin & A.C. Bywater (Eds.), *Modeling Ruminant Digestion and Metabolism in Ruminants*, University of California, Davis, United States of America, pp. 34-42.
- Faichney, G.J., 1986. The kinetics of particulate matter in the rumen. In: L.P. Milligan, W.L. Grovum & A. Dobson (Eds.) *Control of Digestion and Metabolism in Ruminants*. Prentice-Hall, Englewood Cliffs, New Jersey, United States of America, pp.173-195.
- Gill, M., Beever, D.E., & France, J., 1989. Biochemical bases needed for the mathematical representation of whole animal metabolism. *Nutrition Research Reviews* 2, 181-200.

- Hespell, R.B. & Bryant, M.P., 1979. Efficiency of rumen microbial growth: influence of some theoretical and experimental factors on YATP. *Journal of Animal Science* 49, 1640-1659.
- Mertens, D.R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. *Journal of Animal Science* 64, 1548-1558.
- Mulder, M.M., 1988. *Energetic aspects of bacterial growth: a mosaic non equilibrium thermodynamic approach*. PhD Thesis, University of Amsterdam, Amsterdam, The Netherlands.
- Neal, H.D.StC., Dijkstra, J., & Gill, M., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model evaluation. *Journal of Nutrition* 122, 2257-2272.
- Owens, F.N. & Goetsch, A.L., 1986. Digesta passage and microbial protein synthesis. In: L.P. Milligan, W.L. Grovum & A. Dobson (Eds.), *Control of Digestion and Metabolism in Ruminants*, Prentice-Hall, Englewood Cliffs, New Jersey, United States of America, pp. 196- 223.
- Russell, J.B. & Strobel H.J., 1993. Microbial energetics. In: J.M. Forbes & J. France (Eds.) *Quantitative Aspects of Ruminant Digestion and Metabolism*. CAB International, Wallingford, United Kingdom, pp. 165-186.
- Russell, J. B., and R. J. Wallace. 1988. Energy yielding and consuming reactions. In: P.N. Hobson (Ed.), *The Rumen Microbial Ecosystem*. Elsevier Science Publishers, Essex, United Kingdom, pp. 185-215.
- Speckhart, F.H. & Green W.L., 1976. A Guide to Using CSMP—The Continuous System Modelling Program. Prentice-Hall, Englewood Cliffs, New Jersey, United States of America.
- Stouthamer, A.H., 1973. A theoretical study on the amount of ATP required for synthesis of microbial cell material. *Antonie Leeuwenhoek* 39, 545-565.
- Tamminga, S. & Van Vuuren, A.M., 1988. Formation and utilisation of end products of lignocellulose degradation in ruminants. *Animal Feed Science and Technology* 21, 141-159.
- Thornley, J.H.M. & France, J., 1984. Role of modeling in animal production research and extension work. In: R.L. Baldwin & A.C. Bywater (Eds.), *Modeling Ruminant Digestion and Metabolism*, Proceedings 2nd International Workshop. University of California, Davis, United States of America, pp. 4-9.
- Van Vuuren, A.M., Krol-Kramer, F., Van der Lee, R.A. & Corbijn, H.,. 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. *Journal of Dairy Science*, 75, 2215-2225.
- Van Vuuren, A.M., Van der Koelen, C.J. & Vroons-De Bruin, J., 1993. Ryegrass versus corn starch or beet pulp fiber diet effects on digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science*, 76, 2692-2700.

- Wallace, R.J. & Cotta, M.A., 1988. Metabolism of nitrogencontaining compounds. In: P.H. Hobson, (Ed.), *The Rumen Microbial Ecosystem*, Elsevier Science Publishers, Essex, United Kingdom, pp. 217-249.
- Williams, A.G., & Coleman, G.S., 1991. Role of protozoa in the rumen. In: A.G. Williams & G.S. Coleman, Eds.), *The Rumen Protozoa*, Springer-Verlag, New York, United States of America, pp. 317-347.

Appendix

Flow equations in the BA, DA, and DY are given in the same units to facilitate model comparison. The original DA is described in units of moles of N, moles of C, and hours. To express the Michaelis-Menten equations in units similar to those in the DY, in some equations, variables had to be multiplied by 1.257 to substitute moles of protein for moles of N, by 6.0 to substitute moles of carbohydrate for moles of C, by $(14 \times 6.25)/0.51$ to substitute grams of microbial matter for moles of microbial N [Danfær (1990) assumes 51% CP in microbial mass], and by 24 to substitute flows per day for flows per hour. There was no need for such corrections in the BA. Table A1 explains the symbols, notation, and units used in Tables A2 to A5, which present the flow equations and variables for the adaptations performed in the present study.

Table A1. The notation used in the Appendix.

Symbol	Model element	Units
Ac	Acetate	mol
Am	Ammonia	mol
Bu	Butyrate	mol
Ew	Empty BW	kg
Dm	DM	kg
Hx	Rumen-soluble hexose	mol
Ma	Amylolytic microbes	g
Map	Microbial AA and peptides	mol
Mc	Cellulolytic microbes	g
Mcp	Microbial CP	g of N
Mndf	Microbes bound to NDF	g
Msp	Microbes to small particles	g
Mst	Microbes bound to St	g
Ndf	NDF	mol
Ni	N	g of N
Ns	Soluble N	g of N
Nu	Undegradable N	g of N
Pa	Protein available (Ps + Pd)	mol
Pd	Insoluble, degradable protein	mol
Pl	Plasma	
Pr	Propionate	mol
Ps	Soluble protein	mol
Ru	Rumen	
Sc	Dietary soluble carbohydrate	mol
St	Insoluble starch	mol
Ur	Urea	mol

..... continued

<i>Process</i>		
Ab	Absorption	mol/d
Ds	Degradation of insoluble substrate	mol/d
Fs	Fermentation of substrate into VFA	mol/d
Ic	Incorporation of substrate into microbes	mol/d
In	Input to rumen	mol/d or kg/d
Ms	Microbial utilization of substrate (Ic+Fs)	mol/d
Rs	Recycled from microbes to rumen substrate	mol/d
Sp	Saliva production	L/d
<i>Notation</i>	<i>Meaning</i>	
$P_{x,yz}$	Production of x with process y acting on z	mol of x/d
$U_{x,yz}$	Utilization of x with process y acting on z	mol of x/d
C_{mx}	Concentration of microbial mass bound to x	g of Mi/g of x
C_x	Concentration of x	mol of x/L
F_{xy}	Fraction of x in y	g of x/kg of y
V_x	Volume of x	L
xy	Amount of x in y or with process y	mol or kg/d

Table A2. Equations for non-dietary inputs.¹

Flow equation	Units
Model of Baldwin <i>et al.</i> (1987)	
[1] Sp = daily saliva production during eating, ruminating, and resting, respectively = $3.2 \times DmIn + 2.41 \times Ew^{0.75} \times 0.333 + 0.85 \times Ew^{0.75} \times (1 - 0.333)$	L/d
[2] $P_{Am,SpUr} = C_{UrSp} \times Sp \times 2 = 0.007 \times Sp \times 2$	mol of Am/d
[3] $P_{Am,AbUr} = 0.0567 \times Ew^{0.75} / (1 + 0.007/C_{UrPl} + C_{Am}/0.003) \times 2 =$ $[0.0567 \times Ew^{0.75} / (1 + 0.007/0.007 + C_{Am}/0.003)] \times 2$	mol of Am/d
[4] $P_{Ps,SpPs} = 0.002 \times Sp$	mol of Ps/d
Model of Danfær (1990)	
[5] $P_{Ps,SpPs} = 0.0$	mol of Ps/d
[6] $P_{Am,AbUr} = 16.687 \times (UrPl/V_{Pl} - UrRu/V_{Ru}) \times 24 =$ $16.687 \times (2.0479/150 - UrRu/V_{Ru}) \times 24$	mol of Am/d
Model of Dijkstra <i>et al.</i> (1992)	
[7] $Sp = 4.6 + 9.54 \times DmIn + 0.1357 \times F_{Ndf,InDm}$	L/d
[8] $P_{Ps,SpPs} = 0.001 \times Sp$	mol of Ps/d
[9] $P_{Am,AbUr} = [V_{Ru} \times 0.00165 \times F_{Ni,InDm} / (1 + C_{Am}/0.00621)] \times 2$	mol of Am/d

¹ Notation is defined in Table A1.

Table A3. Inclusion of soluble protein pool and removal of urea pool in DA3¹ model.²

Flow equation	Units
Model of Danfær (1990) (equations for rumen passage omitted)	
Input rumen urea, UrRu:	
[1] $P_{Ur,AbUr} = \text{Equation [6] in Table A2}$	mol of Am/d
Output rumen urea, UrRu:	
[2] $U_{Ur,UrAm} = P_{Am,UrAm} = 2.463 \times 24 \times UrRu / (2.723 + UrRu)$	mol of Am/d
Inputs of protein that can potentially be used by microbes, Pa:	
[3] $P_{P,InPa} = (F_{Ni,InDm} - F_{Nu,InDm}) \times DmIn \times 6.25/110.0$	mol of Pa/d
[4] $P_{Pa,RsMap} = 0.157 \times 24 \times Map / (0.0415 + (Map \times 1.257))$	mol of Pa/d
Actual microbial utilization of protein that can potentially be used, Pa:	
[5] $U_{Pa,MuPa} = 1.685 \times 24 \times Pa / (0.92361 + (Pa \times 1.257))$	mol of Pa/d
Model DA3 (equations for rumen passage omitted)	
No rumen urea pool	
Input rumen NH ₃ pool, Am:	
[6] $P_{Am,AbUr} = \text{Equation [9] in Table A2}$	mol of Am/d
Rumen protein pool included for subdivision of potentially available protein for microbes, Pa, into soluble protein, Ps, and insoluble, degradable protein, Pd.	
Input to rumen-insoluble, degradable protein, Pd:	
[7] $P_{Pd,InPd} = (F_{Ni,InDm} - F_{Nu,InDm} - F_{Ns,InDm}) \times DmIn \times 6.25/110.0$	mol of Pd/d
Degradation of rumen-insoluble, degradable protein, Pd:	
[8] $U_{Pd,DsPd} = 1.685 \times 24 \times Pd / (0.92361 + (Pd \times 1.257))$	mol of Pd/d
Inputs to rumen-soluble protein, Ps:	
[9] $P_{Ps,InPs} = F_{Ns,InDm} \times DmIn \times 6.25/110.0$	mol of Ps/d
[10] $P_{Ps,RsMap} = 0.157 \times 24 \times Map / (0.060 + (Map \times 1.257))$	mol of Ps/d
[11] $P_{Ps,DsPd} = U_{Pd,DsPd}$	mol of Ps/d
Microbial utilization of soluble protein, Ps	
[12] $U_{s,MsPs} = 1.685 \times 24 \times Ps / (0.92361 + (Ps \times 1.257))$	mol of Ps/d

¹ Altered version of the model of Danfær (1990).² Notation is defined in Table A1.

Table A4. Equations for absorption of rumen VFA and NH₃.¹

Flow equation ¹	Units
Model of Baldwin <i>et al.</i> (1987)	
[1] $U_{Am,AbAm} = (12.4 - kp_{liq}) \times Am$	mol of Am/d
[2] $U_{Ac,AbAc} = (10.5 - kp_{liq}) \times Ac$	mol of Ac/d
[3] $U_{Pr,AbPr} = (10.5 - kp_{liq}) \times Pr$	mol of Pr/d
[4] $U_{Bu,AbBu} = (10.5 - kp_{liq}) \times Bu$	mol of Bu/d
Model of Danfær (1990)	
[5] $U_{Am,AbAm} = (18.385 \times 24 - kp_{liq}) \times C_{Am}$	mol of Am/d
[6] $U_{Ac,AbAc} = (0.4483 \times 24 - kp_{liq}) \times Ac$	mol of Ac/d
[7] $U_{Pr,AbPr} = (0.4605 \times 24 - kp_{liq}) \times Pr$	mol of Pr/d
[8] $U_{Bu,AbBu} = (0.4540 \times 24 - kp_{liq}) \times Bu$	mol of Bu/d
Model of Dijkstra <i>et al.</i> (1992)	
[9] $U_{Am,AbAm} = V_{Ru}^{0.75} \times 1.097 / (1 + (7.5/pH)7.85) / (1 + 0.0132/C_{Am})$	mol of Am/d
[10] $U_{Ac,AbAc} = V_{Ru}^{0.75} \times 7.86 / (1 + (pH/6.45)6.48) / (1 + 0.338/C_{Ac})$	mol of Ac/d
[11] $U_{Pr,AbPr} = V_{Ru}^{0.75} \times 7.86 / (1 + (pH/6.45)6.48) / (1 + 0.338/C_{Pr})$	mol of Pr/d
[12] $U_{Bu,AbBu} = V_{Ru}^{0.75} \times 7.86 / (1 + (pH/6.45)6.48) / (1 + 0.338/C_{Bu})$	mol of Bu/d

¹ Notation is defined in Table A1.

² pH = daily mean pH, kp_{liq} = fractional liquid passage rate (/d).

Table A5. Incorporation of equations of Dijkstra *et al.* (1992) for degradation of insoluble, degradable starch, NDF, and protein in the BA5 and DA5 models.^{1,2}

Flow equation	Units
Model of Baldwin <i>et al.</i> (1987)	
Original model:	
[1] $U_{St,DsSt} = 6.0 \times St \times C_{Mst}$	mol of St/d
[2] $U_{Ndf,DsNdf} = 9.0 \times Ndf \times C_{Mndf}$	
If ($pH < 6.2$) then	
$U_{Ndf,DsNdf} = [9.0 - (9.0 \times 1.875 \times (6.2 - pH))] \times Ndf \times C_{Mndf}$	mol of Ndf/d
[3] $U_{Pd,DsPd} = 7.0 \times Pa \times C_{Msp}$	mol of Pd/d

Adapted equations in the BA5 model:

- [4] $U_{St, DsSt} = 0.21792 \times Mst \times 100 / (1 + 0.40 / (St / V_{Ru} \times kd_{St}))$ mol of St/d
(multiplication with 100 for comparable results to the DY1 model)
- [5] $U_{Ndf, DsNdf} = 0.16464 \times Mndf \times ((1 - Tf_{63}/24) + (Tf_{63}/24) / (1 + (5.97 / pH-min)^{22.9})) / (1 + 0.40 / (Ndf / V_{Ru} \times kd_{Ndf}))$ mol of Ndf/d
- [6] $U_{Pd, DsPd} = 0.0576 \times Msp \times 10 / (1 + 0.40 / (Pa / V_{Ru} \times kd_{Pd}))$ mol of Pd/d
(multiplication with 10 for comparable results to the DY1 model)

Model of Danfær (1990):

No distinction between soluble and insoluble substrate in the original model

Microbial incorporation of carbohydrates:

- [7] $U_{Sc, IcSc} = 0.073 \times 24 \times Sc$ mol of Sc/d
- [8] $U_{St, IcSt} = 0.073 \times 24 \times St$ mol of St/d
- [9] $U_{Ndf, IcNdf} = 0.073 \times 24 \times Ndf$ mol of Ndf/d

Microbial fermentation of carbohydrates:

- [10] $U_{Se, FsSc} = 1.0534 \times 24 \times Mcp \times (Sc + St + Ndf) / (kf + (Sc + St + Ndf) \times 6) \times Sc / (Sc + St) \times \exp(-0.04 \times Ndf / (Sc + St))$ mol of Sc/d
- [11] $U_{St, FsSt} = 1.0534 \times 24 \times Mcp \times (Sc + St + Ndf) / (kf + (Sc + St + Ndf) \times 6) \times St / (Sc + St) \times \exp(-0.04 \times Ndf / (Sc + St))$ mol of St/d
- [12] $U_{Ndf, FsNdf} = 1.0534 \times 24 \times Mcp \times (Sc + St + Ndf) / (kf + (Sc + St + Ndf) \times 6) \times (1 - \exp(-0.04 \times Ndf / (Sc + St)))$ mol of Ndf/d
kf initially 2.50,
If $(Sc + St + Ndf) < 30.25/6$ mol of carbohydrate then $kf = kf + 0.1$
If $(Sc + St + Ndf) > 40.00/6$ mol of carbohydrate then $kf = kf - 0.1$

In the DA5 version of the model of Danfær (1990) the pool of soluble carbohydrate in the rumen, Hx, was considered to be a rumen collection pool for dietary soluble carbohydrates (Sc), degraded St, and degraded Ndf. Degradation equations of Dijkstra *et al.* (1992) were applied for the description of input flows from St and Ndf to Hx, whereas identical equations to the original model of Danfær (1990) were applied for microbial incorporation and fermentation as output flows from Hx.

Degradation of insoluble carbohydrates:

- [13] $U_{St, DsSt} = 0.21792 \times Mcp \times ((14 \times 6.25) / 0.51) / (1 + 0.40 / (St / V_{Ru} \times kd_{ST}))$ mol of St/d
- [14] $U_{Ndf, DsNdf} = 0.16464 \times Mcp \times ((14 \times 6.25) / 0.51) / ((1 - Tf_{63}/24) + (Tf_{63}/24) / (1 + (5.97 / pH-min)^{22.9})) / (1 + 0.40 / (Ndf / V_{Ru} \times kd_{Ndf}))$ mol of Ndf/d

Microbial utilization of rumen soluble carbohydrates:

- [15] $P_{Hx, DsDm} = U_{St, DsSt} + U_{Ndf, DsNdf} + P_{Sc, InSc}$ mol of Hx/d
- [16] $U_{Hx, IcHx} = 0.073 \times 24 \times Hx$ mol of Hx/d
- [17] $U_{Hx, FsHx} = 1.0534 \times 24 \times Mcp \times Hx / (kf + Hx \times 6)$ mol of Hx/d
kf initially 2.50,
If $(Hx + St + Ndf) < 30.25/6$ mol of carbohydrate, then $kf = kf + 0.1$

If $(Hx + St + Ndf) > 40.00/6$ mol of carbohydrate, then $kf = kf - 0.1$

Degradation of insoluble protein (substituted for Equation [8] & [11] in Table A3):

$$[18] \quad U_{Pd, DsPd} = 0.0576 \times Mcp \times ((14 \times 6.25)/0.51) / (1 + 0.40 / (Pd/V_{Ru} \times kd_{Pd})) \quad \text{mol of Pd/d}$$

Model of Dijkstra *et al.* (1992):

$$[19] \quad U_{St, DsSt} = 0.21792 \times Ma / (1 + 0.40 / (St/V_{Ru} \times kd_{St})) \quad \text{mol of St/d}$$

$$[20] \quad U_{Ndf, DsNdf} = 0.16464 \times Mc / ((1 - Tf_{63}/24) + (Tf_{63}/24) / (1 + 5.97/pH-min)^{22.9}) / (1 + 0.40 / (Ndf/V_{Ru} \times kd_{Ndf})) \quad \text{mol of Ndf/d}$$

$$[21] \quad U_{Pd, DsPd} = 0.0576 \times (Ma + Mc) / (1 + 0.40 / (Pd/V_{Ru} \times kd_{Pd})) \quad \text{mol of Pd/d}$$

¹ pH = mean pH; $pH-min$ = minimum pH, Tf_{63} = time interval pH < 6.3 (h); kd_{St} , kd_{Ndf} , and kd_{Pd} = *in situ* determined fractional degradation rates (/d) of insoluble, degradable St, Ndf and Pd, respectively; kf = Michaelis-Menten constant for carbohydrate fermentation rate (mol of C), BA5 = altered version of Baldwin *et al.* (1987) model, and DA5 = altered version of the Danfær (1990) model.

² Notation is defined in Table A1.

Table A6. Molecular weights and fractions of glycerol and fatty acids in dietary lipid.

	Model ¹		
	BA	DA	DY
	(g/mol of carbohydrate or AA)		
Dietary soluble carbohydrates	171	171	162
Starch	162	162	162
Hemi-cellulose	132	162	162
Cellulose	162	162	162
Dietary soluble protein	115	110	110
Insoluble protein	115	110	110
Rumen-soluble carbohydrate	180	162	162
Rumen-soluble amino acids	133	110	110
	(g/g)		
Ratio of glycerol to fatty acids	138:862	105:895	138:862

¹BA = model of Baldwin *et al.* (1987), DA = model of Danfær (1990), and DY = model of Dijkstra *et al.* (1992).

Table A7. Coefficients for VFA production with substrate fermentation for diets containing >55% roughage.

Substrate	Acetate			Propionate			Butyrate			ATP ¹		
	BA ²	DA	DY ³	BA	DA	DY	BA	DA	DY	BA	DA	DA
SC	1.38	1.08	1.38	0.40	0.27	0.41	0.11	0.32	0.10	4.0	4.61	4.61
ST	1.20	1.16	1.19	0.34	0.54	0.28	0.23	0.15	0.20	4.0	4.52	4.52
Hemi-cellulose	1.14	1.09	1.13	0.40	0.73	0.38	0.23	0.09	0.21	4.0	4.22	4.22
Cellulose	1.32	1.09	1.32	0.20	0.73	0.17	0.24	0.09	0.23	4.0	4.22	4.22
Protein	0.60	0.71	0.40	0.60	0.22	0.13	0.25	0.11	0.08	0.97	0.0	0.0

¹ Dijkstra *et al.* (1992) did not explicitly describe ATP balance or ATP coefficients.

² Argyle & Baldwin (1988) made VFA coefficients with sugar and starch fermentation dependent on rumen pH according to the following equations: If (pH < 6.2) and (pH > 5.4) then acetate from dietary soluble carbohydrate (SC) = $0.70 + ((pH - 5.4)/0.8) \times (1.38 - 0.70)$, propionate from SC = $0.50 + ((pH - 5.4)/0.8) \times (0.40 - 0.50)$, butyrate from SC = $0.40 + ((pH - 5.4)/0.8) \times (0.11 - 0.40)$, acetate from starch (ST) = $0.66 + ((pH - 5.4)/0.8) \times (1.20 - 0.66)$, propionate from ST = $0.82 + ((pH - 5.4)/0.8) \times (0.34 - 0.82)$, butyrate from ST = $0.26 + ((pH - 5.4)/0.8) \times (0.23 - 0.26)$. BA = Model of Argyle & Baldwin *et al.* (1988), DA = Model of Danfær (1990), and DY = model of Dijkstra *et al.* (1992).

³ The valerate fraction of VFA carbon is not considered and thus is missing with the DY.

Chapter 4

Comparison and Evaluation of Mechanistic Rumen Models

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Comparison and Evaluation of Mechanistic Rumen Models

Abstract

Mechanistic rumen models of Baldwin (1995), Danfær (1990) and Dijkstra *et al.* (1992) were compared on identical inputs that were derived from trials with lactating dairy cows fed on grass herbage. Consistent differences were detected between models and between predicted and observed outputs. None of the models seemed to predict all nutrient flows best. The models particularly differed in the representation of microbial metabolism: degradation of insoluble substrate, fermentation of substrate into volatile fatty acids, and incorporation of substrate into microbial matter. Differences amongst models in the prediction of these processes compensated for each other and consequently all models predicted the duodenal flow of non-NH₃ N, microbial N and organic matter reasonably well. Large differences remained in the prediction of individual nutrient flows, however, and it was stressed that in order to enhance prediction of the profile of nutrient flows, the mechanisms of microbial metabolism need to be tested on their ability to describe the intra-ruminal transactions. However, this requires more-detailed information on individual nutrient flows and on the microbial or non-microbial origin of duodenal contents. Parameter inputs for physical and chemical feed properties were identified that are improperly defined in extant models or susceptible to error. The description of these feed characteristics needs to be developed further and become identifiable for a wide range of dietary conditions.

Introduction

Current research into the nutrition of lactating dairy cows focuses on the profile of nutrients absorbed from the digestive tract, on the partitioning of these nutrients in intermediary metabolism, and on the utilization of these nutrients for milk production and body tissues. However, when theories of dietary influences on nutrient partitioning and animal production are to be investigated, the effect of dietary factors on feed digestion and the profile of nutrients absorbed from the gastrointestinal tract needs to be defined. In particular, an understanding of rumen function is essential for estimating these nutrient flows, because the profile of nutrients can change dramatically due to events in the rumen.

Mathematical modelling is an important instrument for understanding and integrating knowledge (Baldwin, 1995). Detailed dynamic models that describe the mechanism of rumen function in cattle have been developed by Baldwin *et al.* (1987; BA), Danfær (1990; DA) and Dijkstra *et al.* (1992; DY). Although the BA and DA models are whole-animal models, in contrast to the DY model, the part that describes rumen function can easily be extracted in

both cases because it is formulated as a separate module. Although the models have differing objectives and consequently apply different concepts and theories, all three models basically simulate the digestion, absorption and outflow of nutrients from the rumen. The model inputs required include daily feed intake, the composition and other characteristics of the feed, and several parameters describing rumen conditions. The BA model describes a mechanism to explain the rate at which nutrients become available for the micro-organisms by including a representation of particle dynamics and the physical relationships between particulate substrate and micro-organisms. The DA model describes several pools for the constituents of the single type of micro-organism represented, whereas the DY model is concerned with studying the interactions between distinct microbial groups and with a more detailed description of fermentation processes. Extensive evaluations, by comparing observed with predicted values, have been published for the DY model (Neal *et al.*, 1992).

An essential step in the evolution of rumen modelling is to investigate whether specific aspects of rumen function are represented satisfactorily in extant models and to evaluate the importance of the different concepts and theories used in explaining observed rumen function quantitatively. However, an integral comparison between the BA, DA and DY models is hindered because, for example, differences in model behaviour appearing from previous evaluation studies are confounded by the use of different sets of evaluation data with each rumen model, or, extensive evaluations have not been published. To overcome such limitations in the present study, the BA, DA and DY models were evaluated and compared on the same observations of rumen function. The objective was to compare and evaluate the different concepts adopted in these models on inputs that correspond between models and coincide with experimental rumen conditions. Results of feeding trials with lactating dairy cows that provided almost all the required inputs and many data for evaluation of model predictions were used.

Materials and methods

Selected data for evaluation

Experiments of Van Vuuren *et al.* (1992, 1993) with lactating dairy cows, fed on fresh grass, were selected for the evaluation of model predictions. The experiments were selected because of the extensive determination of rumen kinetics from measurements of duodenal flows, rumen evacuations, *in situ* degradation characteristics, diet analyses and endproducts of fermentation (volatile fatty acids (VFA), NH₃ and microbial matter). The available data provided almost all feed characteristics and parameter inputs that were required by the BA, DA and DY models. Considerable variation existed in these model inputs (Table 1) and in observed values on which model predictions were evaluated.

Animals were fed on freshly cut ryegrass (85% *Lolium perenne*), which accounted for at least half of the total diet. Models have not been evaluated on fresh grass before. Although the sum of the chemical fractions was less than 100 % of total DM, the model inputs were not corrected because the composition of missing DM was not clear.

In the first experiment (Van Vuuren *et al.*, 1993), the effect was studied of partial replacement of ryegrass herbage (diet 1) by maize meal plus hominy feed (diet 2) and sugarbeet pulp (diet 3) on rumen digestion and intestinal amino acids (AA) in lactating dairy cows. Ryegrass comprised 890, 570 and 560 g/kg total DM of diets 1, 2 and 3 respectively, and DM intakes were 16.2, 16.3 and 16.5 kg/d respectively, including 1.7 kg commercial concentrate/d. Sugarbeet pulp contains a considerable proportion of pectin which is highly degradable in the rumen but not identified by chemical feed analysis (Van Vuuren *et al.* 1993). This additional missing fraction compared with chemical analysis of the other experimental feedstuffs was corrected with an extra 80 g soluble carbohydrate/kg DM intake in diet 3. Further, it was assumed that 25% of soluble N (e.g. nucleic acids and nitrate) was immediately hydrolysed to NH₃ in the rumen (Goswami & Willcox, 1969). Consequently, all remaining model inputs of feed N were considered to consist of amino acid N (AAN). Because the major part of soluble feed N was of herbage origin, these assumptions were applied to total diets. Rumen pool sizes and duodenal flows of microbial N (MN) were estimated with diaminopimelic acid (DAPA) as microbial marker (DAPA method). The microbial fraction in the duodenal flow of AAN was estimated with the observed fraction of MN in duodenal flow of non-NH₃ N (NAN).

The second experiment (Van Vuuren *et al.*, 1992) studied effects of N fertilization and season: 500 (diets 4 and 7) vs. 275 kg N/ha per year (diets 5 and 6), harvested either in June-July (diets 4 and 5) or September-October (diets 6 and 7). DM intake varied between 13.0 and 16.8 kg/d and included 1 kg commercial concentrate/d. The same assumptions were made about N inputs as for the first experiment. Microbial protein was quantified by two methods. First, the proportion of microbial AAN (MAAN) in the duodenal flow of total AAN was estimated from measured AA profiles in grass, isolated microbial matter and duodenal contents (AA profile method). Second, rumen pool size and duodenal flow of MN were estimated with the DAPA method (results not published). With both methods the observed microbial proportion was applied to estimate MN flow from the duodenal flow of NAN as well as MAAN flow from the duodenal flow of AAN.

Rumen models

The BA, DA and DY models were written in the Continuous System Modeling Program (CSMP; Speckhart & Green, 1976). Simulations were performed with a fourth-order variable step length Runge-Kutta as integration method and with integration periods for the BA, DA and DY models of 20, 50 and 20 d respectively, which proved to be sufficient to reach a stable steady-state solution in every condition. Models were evaluated and compared

Table 1. Inputs to the models of Baldwin (1995; BA), Danfær (1990; DA) and Dijkstra *et al.* (1992; DY) for seven test diets

	Diet							Model ¹		
	1	2	3	4	5	6	7			
	Chemical composition of diet (g/kg DM)									
SC	146	104	110	164	174	146	193	BA	DA	DY
DST ²	6	121	18	4	3	4	3	BA	DA	DY
SST ^{2,3}	2	36	5	1	1	1	1	BA	DA	DY
NDF	409	351	407	373	397	406	333	BA	DA	DY
DNDF ²	373	326	376	345	360	364	311	...	DA	DY
Lignin	12	10	13	13	14	27	12	BA
N	28.9	27.1	25.8	33.6	27.9	27.1	31.3	BA	DA	DY
SN ²	5.6	8.5	6.5	16.8	12.4	10.1	14.0	BA	...	DY
UN ²	1.1	1.2	1.4	1.1	1.4	1.6	1.1	...	DA	DY
NH ₃	1.4	2.1	1.6	4.2	3.1	2.5	3.5	BA	DA	DY
Lipid	39	41	31	37	37	41	37	BA	DA	DY
	Parameter inputs ⁴									
Intake, kg/d	16.2	16.3	16.5	13.3	16.8	13.0	15.2	BA	DA	DY
kd_{DNDF}^2 , /d	1.25	1.56	1.65	1.41	1.24	1.10	1.64	DY
kd_{DP}^2 , /d	1.97	2.08	1.87	2.12	2.17	1.53	2.14	DY
kd_{DST}^2 , /d	3.00	2.71	3.00	3.00	3.00	3.00	3.00	DY
kp_{liq} , /d	3.72	3.89	3.89	4.51	4.13	2.86	2.26	BA	...	DY
kp_{OM} , /d	0.77	1.22	0.94	0.79	0.82	0.58	0.58	BA	...	DY
pH	6.14	6.16	6.15	6.63	6.12	6.20	6.13	BA	...	DY
$pH-min$	5.78	5.87	5.86	6.40	5.90	6.00	5.95	DY
Tf_{63} , h	17.0	14.0	15.5	0.0	20.0	15.0	18.0	DY
Tf_{60} , h	5.0	4.5	4.5	0.0	4.0	2.0	4.0	DY
Vol , L	64.5	53.8	57.7	54.6	64.0	67.8	72.9	BA	DA	DY

SC, soluble carbohydrate; DST, insoluble, degradable starch; SST, soluble starch NDF, neutral-detergent fibre; DNDF, degradable NDF; SN, soluble nitrogen; UN, undegradable N; kp_{liq} , fractional liquid passage rate; kp_{OM} , fractional organic matter passage rate; kd_{DNDF} , degradation rate NDF; kd_{DP} , degradation rate protein; kd_{DST} , degradation rate starch; pH , mean pH; $pH-min$, minimum pH; Tf_{63} , time interval pH < 6.3; Tf_{60} , time interval pH < 6.0; Vol , mmen non-dry matter.

¹ Indicates whether a model input is used (BA, DA or DY) or not used (. . .) in a model.

² Value estimated from nylon bag incubations.

³ With the exception of diet 2, starch solubility was assumed to be 20 %.

⁴ All remaining parameter inputs are described in the Material & methods.

on their steady-state solution. Certain parameter inputs for the BA and DA models were not measured, although their value clearly depends on the diet fed.

BA model. An updated version of the BA model was used (Baldwin, 1995). The experimental database delivered all required inputs for the BA model, except those for particle dynamics in the rumen. Although Baldwin *et al.* (1987) considered these parameter inputs to be variable, they used constants for the description of rumen particle dynamics: fractional outflow rates of small particles and fluid of 1.45 /d and 3.5 /d respectively, a fractional comminution rate of large particles of 4.5 /d, and little variation of the partitioning of ingested particles over the large and small particle pool (i.e. a particle size factor of 0.4 or 0.5). These constant parameter values resulted in little variation in predicted outflow rate of organic matter (OM) of 0.69 (SD 0.03) /d (results not shown). However, the observed fractional outflow rates of OM varied much more among diets: 0.81 (SD 0.20) /d. To account for such variation of particle dynamics in the present study, it was necessary to vary outflow rates of OM and liquid. Moreover, if observed values are used for particle dynamics with the DA and DY models, it is a prerequisite to use them also with the BA model for model comparison on identical inputs.

In the BA model, fractional outflow rate of particles only applies to the small-particle pool, whereas observed outflow rates of particulate OM apply to both small and large particles. Therefore, the observed fractional outflow rates of OM were corrected by the factor (total particle pool):(small particle pool). This correction is only valid with accurate prediction of the pool sizes of small and large particles in steady-state. To predict pool sizes, the input parameters of fractional comminution rate of large particles and particle size factor must be considered (Baldwin *et al.* 1987). However, these key parameters in model prediction were not measured and, therefore, appropriate estimates had to be made.

The comminution rate of large particles is formulated as a rumination factor x the fractional comminution rate of large particles (Baldwin *et al.* 1987). In the BA model the rumination factor is 0.333 in steady-state. With a fractional comminution rate of 4.50 /d this results in a comminution rate of 1.5 /d. Applying the corrected estimates of outflow rate of OM to the small-particle pool in the BA model, with 0.4 for the particle size factor and 4.5 /d for the fractional comminution rate, resulted in large prediction errors. Second, these parameter values resulted in similar pool sizes for small and large particles, although experimental data (Poppi *et al.*, 1981; Bosch, 1991) of rumen contents clearly show predominance of small particles (72-81%) for the type of diets used in this study. Therefore, the fractional comminution rate and the particle size factor were re-estimated.

Experimental estimates of the comminution rates of large particles range from 0.96 to 3.12 /d (Woodford & Murphy, 1988). Although techniques used in several studies with lactating dairy cows were different, reported estimates affirm such a large range (McLeod & Minson, 1988; Woodford & Murphy, 1988; Bosch, 1991). Sensitivity analysis (Baldwin *et al.*, 1987) demonstrated that, in particular, inappropriately low estimates of fractional

comminution rates can impair model prediction, although effects remain smaller with high-quality diets. Considering the low fibre content and the very high rumen digestibility of the diets (91.8 (SD 1.2) % neutral-detergent fibre (NDF) degradability in sacco and 75.8 (SD 5.4) % rumen NDF digestion *in vivo*; Van Vuuren et al. 1992, 1993), the fractional comminution rate was increased from 4.5 /d to 8.5 /d. The resulting comminution rate in the model ($8.5 /d \times 0.333 = 2.84 /d$) approaches the highest estimates reported in literature.

Estimates of the particle size factor are much more difficult to obtain, because it not only represents the proportion of small particles in the diet but also considers the apparent availability properties of particles in the rumen (Baldwin *et al.*, 1987). Because diets consisted largely of fresh perennial ryegrass harvested at an early stage of maturity, a particle size factor of 0.6 was taken instead of 0.4 or 0.5 assumed by Baldwin *et al.* (1987).

DA model. All model inputs for the DA model could be derived from the experimental data, except for the so-called *G* parameter that partitions total carbohydrate fermentation into that of soluble carbohydrates, starch, or NDF, and has a value between 0.0 and 1.0. Danfær (1990) considered *G* to be dependent on the diet fed and calibrated a value of 0.0006 on a reference diet. Such small values always cause a model response of almost no escape of starch (Danfær, 1990), although *in vivo* duodenal flow of starch may increase considerably with increasing starch content in the diet (Nocek & Tamminga, 1991). Danfær (1990) probably encountered no inaccuracies in predicted duodenal flow of starch because his reference diet contained very little starch (30 g/kg). Another consequence of such a small *G* value is that the model responds with a decreased duodenal flow of rapidly fermentable carbohydrates to an increased content of these carbohydrates in the diet (Danfær, 1990), irrespective of other diet characteristics. These model responses were considered incorrect and *G* was arbitrarily increased to a high value of 0.4, which enabled the model to simulate a substantial duodenal flow of starch with increased starch content in a diet.

The DA model does not distinguish between rumen pools of soluble and insoluble substrate. Therefore, it was assumed that the fractional outflow rate of rumen substrates is determined by observed fractional outflow rates of both fluid and OM, as described previously (Danfær, 1990). Because microbes flow out of the rumen with both particles and fluid, the fractional outflow rate of microbial matter was assumed to be half the observed fractional outflow rate of fluid (Danfær, 1990). This assumption allowed for variation in outflow rate of microbial matter with observed values, yet estimates still agreed reasonably with Danfær's original estimate.

DY model. Experimental data delivered all required inputs for the DY model. Observed fractional outflow rates of fluid and OM were used as estimates for the model parameters of fluid and particle outflow respectively.

Model predictions

Predicted rumen pool sizes and duodenal flows were compared amongst models and evaluated with measured values. The prediction error was calculated as the square root of the mean squared differences between predicted and observed values, expressed as a percentage of the observed mean. Considering the large standard deviation of the experimental data (Van Vuuren *et al.*, 1992, 1993), a 10% deviation as a criterion for prediction accuracy seems reasonable and predictions were declared accurate at a prediction error < 10%. Other predictions (fermentation of substrate into VFA and incorporation of substrate into microbial matter) could only be compared amongst models, because no experimental data were available.

Results

Model responses to changes in input were similar to observed responses for duodenal flows of NAN, AAN, MAAN, MN, starch, NDF, lipids and OM. However, consistent deviations existed amongst models and between predicted and observed values. Duodenal flows of NAN and OM were predicted accurately by all three models (Figure 1A). Rumen pools were most accurately predicted by the BA model, with the smallest prediction errors for NAN, MN, NDF and OM (Figure 1B). Intra-ruminal transactions and duodenal flows of nutrients will be discussed in the following section.

Rumen transactions of substrates

Rumen transactions and duodenal flow of organic matter. The models differed in predicted amounts of OM either fermented into VFA or incorporated into microbial mass (Tables 2 and 3). Prediction errors of duodenal flow of OM remained small with every model (Figures 1A & 2), despite their different predictions of the amount of fermented OM (i.e. OM not appearing in the duodenum).

Rumen transactions of carbohydrates. Soluble and degraded carbohydrates are either fermented into VFA, incorporated into microbial mass, or escape from the rumen without being used by micro-organisms. On average, fermentation accounted for 68, 57 and 53%, incorporation for 13, 20 and 32%, and escape for 19, 22 and 15% of carbohydrate input with the BA, DA and DY models respectively (Table 2). Differences amongst models in predicted amounts of carbohydrate fermented and incorporated partially compensated for each other. The predicted amount of carbohydrate available for microbial use was highest in the DA and

DY models because they had the highest sum of degraded insoluble starch and NDF (Table 2). A different order was predicted with the BA and DA models for the amount of carbohydrate actually utilized by microbes as apparent from predicted escape of feed carbohydrates.

Duodenal flows of carbohydrate. The DA and DY models underpredicted the duodenal flow of NDF (Figures 1A & 3A). The DA model underpredicted the duodenal flow of starch of the high-starch diet (diet 2), whereas the DY and BA models predicted more accurately, although still deviating from the observed values (Figure 3B). On the other hand, the DA model gave more accurate predictions of duodenal starch flow on total herbage diets (diets 1, 4, 5, 6 and 7), in contrast to over- and underprediction with the BA and DY models respectively. The BA model predicted the largest duodenal flow of starch because of the highest starch content in microbial matter (on average, the microbial fractions in duodenal flow of starch were 210, 40 and 110 g/kg in the BA, DA and DY models respectively). Predictions of the duodenal flow of soluble carbohydrate could not be evaluated because no experimental data were available. Large duodenal flows predicted by the DA and DY models contrasted with low duodenal flows predicted by the BA model (Figure 3C).

Because the excess duodenal flow of soluble carbohydrate in the DY and DA models (Figure 3C) compensated for the lower duodenal flows of NDF and starch (Table 2), the models predicted a comparable duodenal flow of carbohydrate (on average, 250, 230 and 180 g/kg of the rumen input of feed carbohydrate with the BA, DA and DY models respectively).

Rumen transactions and duodenal flow of lipid. The BA model predicted duodenal flow of lipid accurately (Figures 1A & 3D). The DA and DY models overpredicted because the assumed lipid content in microbial matter and the lipid utilization by microbes were lower than in the BA model.

Rumen transactions of nitrogen. The DA model predicted the highest degradation of feed AAN. Soluble and degraded AAN is either incorporated into microbial mass, fermented into VFA and NH₃ or escapes from the rumen. Differences amongst models in fermented AAN were opposite to differences in incorporated feed AAN (on average there was 64, 50 and 56% fermentation, and 14, 35 and 23% incorporation of the rumen input of feed AAN with the BA, DA and DY models respectively; Table 3). Considerable differences remained amongst models in the predicted escape of feed N to the duodenum.

Duodenal flows of nitrogen. The BA and DY models predicted the duodenal flow of NAN accurately, whereas the DA model underpredicted (Figures 1A & 4A). However, the

microbial contribution to duodenal NAN appeared to be overpredicted in all three models (Figures 1A & 4C).

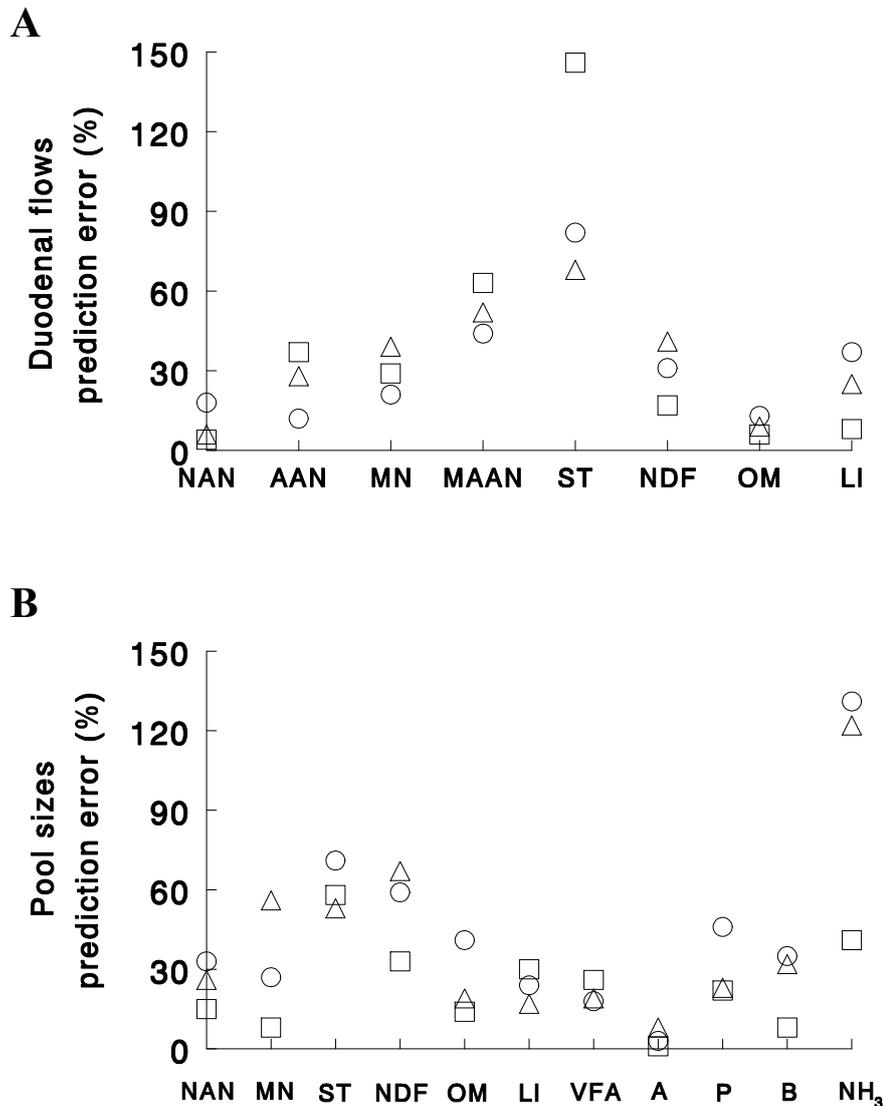


Figure 1. Prediction errors of (A) duodenal flows and (B) rumen pools on seven test diets by the models of Baldwin (1995; □), Danfær (1990; ○) and Dijkstra *et al.* (1992; △). Errors were calculated as square root of mean squared differences between predictions and observations, expressed as a percentage of the observed mean. AAN, amino-acid N; A, molar percentage of acetate; AM, concentration of ammonia; B, molar percentage of butyrate; LI, lipids; MAAN, microbial amino-acid N; MN, microbial N; NAN, non-ammonia N; NDF, neutral-detergent fibre; OM, organic matter; P, molar percentage of propionate; ST, starch; VFA, concentration of volatile fatty acids. Prediction errors were calculated from duodenal flows in g/d or g N/d or from rumen pools in g or g N, except for VFA and AM in mmol/l and molar percentages of VFA in %.

Table 2. Predicted duodenal flows and intra-ruminal transactions of carbohydrates by the models of Baldwin (1995; BA), Danfær (1990; DA) and Dijkstra *et al.* (1992; DY) for seven test diets.

	Diet						
	1	2	3	4	5	6	7
	Duodenal flows (g/d)						
Feed CH ₂ O ¹							
BA	1654	3322	2223	1127	1829	1021	934
DA	1771	2254	2317	2072	2157	1577	1470
DY	1435	1810	1415	1074	1662	1038	748
Microbial starch							
BA	524	490	551	469	560	420	478
DA	101	108	101	153	84	93	128
DY	252	435	363	192	297	193	330
Microbial remains ²							
BA	295	279	317	266	321	238	270
DA	914	972	914	1382	762	845	1166
DY	709	660	726	551	706	497	540
	Transactions (g/d)						
Degraded insoluble CH ₂ O							
BA	5116	4257	4827	3918	4927	4332	4195
DA	5668	6574	5782	3986	5516	4380	4349
DY	5693	6305	6042	4455	5573	4529	4520
Fermented CH ₂ O							
BA	6350	5624	6769	5003	6583	5324	6053
DA	5950	5877	6054	3329	5893	4181	4795
DY	4846	5216	5674	3863	5058	3931	4487
Incorporated CH ₂ O ³							
BA	1207	1136	1315	1134	1327	968	1147
DA	1477	1937	1926	1853	1677	1544	1858
DY	2930	3056	3218	2327	3019	2344	2899

¹ CH₂O, total of solubilized carbohydrates, starch and neutral-detergent fibre.

² Microbial organic matter – microbial N × 6.25 – microbial starch.

³ CH₂O input – fermented CH₂O – duodenal flow of feed CH₂O.

Assuming feed non-AA NAN is input to the rumen NH₃ pool, predicted duodenal flows of protein were considered to consist of AAN only. Duodenal flow of NAN minus MN and duodenal flow of AAN minus MAAN provide estimates of feed N and feed AAN respectively, that escapes from the rumen. Comparison of these calculations on experimental

data and model predictions (Table 3) shows that the duodenal flow of non-AA NAN (e.g. nucleic acids) is strongly underpredicted by all models. On average, the BA, DA and DY models predicted 81, 57 and 80% of observed duodenal flow of feed N, 136, 93 and 132% of feed AAN, and 30, 38 and 65% of non-AA NAN respectively (prediction errors of feed N 18, 47 and 25%, of feed AAN 38, 24 and 27%, and of non-AA NAN 71, 62 and 36% with the BA, DA and DY models respectively; Table 3).

Products of microbial substrate utilization

Fermentation end-products. Because VFA production was not measured, models could only be evaluated on the concentration and molar percentage of VFA. Differences between models in the predicted production of VFA were inverted in the predicted concentrations because of different absorption rates. Although the BA model predicted the largest VFA production, it underpredicted rumen concentration of VFA (Figure 5A) with the largest prediction error (Figure 1B). Model predictions of the molar percentage of individual VFA deviated strongly from the observed percentages (Figures 1B, 6A, 6B & 6C). Only the DY model includes the formation of valerate (overpredicted with a prediction error of 515%, results not shown). This extra VFA influences VFA predictions, because attribution of the C in valerate to acetate, propionate, or butyrate would increase the VFA production and alter the molar percentages. NH₃ is an end-product of protein fermentation in addition to VFA and predicted and observed rumen concentrations of NH₃ differed strongly (Figures 1B & 5B). In particular the response of the BA and DY models (except on diet 7 with the DY model) to input variation corresponded to the observed response in concentration of NH₃.

Microbial synthesis. The observed duodenal flows of MN and MAAN on diets 4-7 were much lower with the AA profile method than with the DAPA method (Figures 4C & 4D). Because results with the DAPA method were considered to be more accurate, for reasons that will be discussed, model predictions were evaluated only on observations with the DAPA method. The models predicted similar duodenal flows of MN (Figures 4C & 4D). Consequently, differences amongst models in the predicted incorporation of AAN (26, 77 and 45% of duodenal flow of MN with the BA, DA and DY models respectively) compensated with opposite differences in net MN synthesis from NH₃ (78, 36 and 58% of duodenal flow of MN respectively). In contrast to the DY model, the assumed AAN content in MN seemed to be too high in the BA and DA models because duodenal flow of MAAN was more overpredicted than that of MN (Figures 4C & 4D). Further, absolute overpredictions of the duodenal flow of MAAN and AAN were of a similar size in the BA and DY models (Figures 4B & 4D). This is illustrated by average prediction errors of the duodenal flow of MAAN vs. AAN in the BA, DA and DY models of 40 vs. 37, 27 vs. 12, and 31 vs. 28% respectively (Figure 1A).

Table 3. Observed (Exp) and predicted duodenal flows and intra-ruminal transactions of nitrogen fractions by the models of Baldwin (1995; BA), Danfær (1990; DA) and Dijkstra *et al.* (1992; DY) for seven test diets

	Diet						
	1	2	3	4	5	6	7
	Duodenal flows (g N/d)						
Feed N ¹							
Exp	154	164	154	87	116	72	85
BA	126	150	125	75	90	54	61
DA	61	66	60	67	76	51	58
DY	92	128	95	94	92	63	73
Microbial N							
Exp	255	260	242	220	255	190	224
BA	264	247	277	236	282	212	240
DA	271	274	278	158	274	179	201
DY	315	295	323	247	315	222	242
Feed AAN ¹							
Exp	80	97	78	55	90	36	68
BA	127	150	123	76	91	55	60
DA	61	67	60	67	75	51	58
DY	92	129	94	94	92	63	74
Microbial AAN							
Exp	172	176	163	152	171	124	156
BA	226	212	238	202	242	181	206
DA	217	219	223	127	220	143	162
DY	227	212	233	178	227	160	174
Non-AAN NAN							
Exp	157	151	155	100	110	102	85
BA	37	35	41	33	39	30	35
DA	54	54	55	31	55	36	39
DY	88	82	91	69	88	62	67
	Transactions (g/d)						
Degraded AAN							
BA	253	156	198	152	173	168	204
DA	414	372	371	357	374	299	410
DY	337	244	271	196	222	187	232
Fermented AAN							
BA	267	202	215	272	268	230	331
DA	183	191	190	198	193	185	245
DY	248	184	204	214	221	203	304

.... Continued

Incorporated AAN ²							
BA	60	64	70	51	67	42	38
DA	204	153	152	129	152	86	124
DY	114	107	110	91	112	60	52
Net MN synthesis from NH ₃ ³							
BA	204	183	207	185	215	170	202
DA	67	121	126	29	122	93	77
DY	201	188	213	156	203	162	190

AAN, amino acid N; MN, microbial N; NAN, non-ammonia N.

¹ Feed AAN = total AAN – microbial AAN; feed N = NAN – microbial N.

² Input AAN - duodenal flow of feed AAN – fermented AAN.

³ Duodenal flow of MN – incorporated AAN.

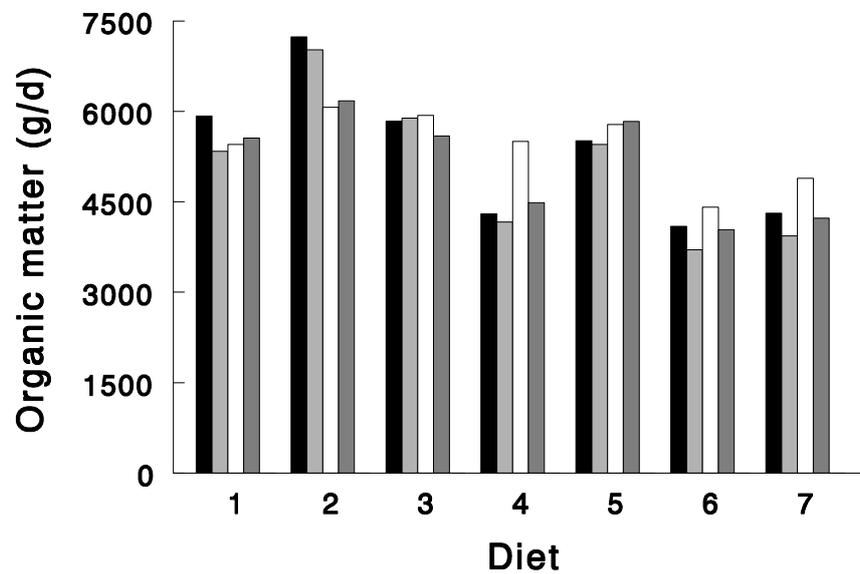


Figure 2. Comparison of observed (■) and predicted values of duodenal flow of organic matter on seven test diets by the models of Baldwin (1995; ■), Danfær (1990; □) and Dijkstra *et al.* (1992; ■).

Discussion

Input parameters

The necessity to estimate some unknown parameter inputs in the present study is inherent to the use of these rumen models and should not be considered as model adjustment. The model-builders clearly stated that these parameters are not internal model parameters but required inputs to the model that are diet-specific and need to be estimated (Baldwin *et al.*, 1987; Danfær, 1990; Dijkstra *et al.*, 1992). The builders also had to estimate appropriate values for lack of experimental data, although it was not always described how those parameters were estimated or should be estimated (Baldwin *et al.*, 1987; Danfær, 1990). For example, if originally published values were used for the particle comminution rate and the particle size factor in the BA model, a duodenal flow of NDF of 3.2 kg/d was predicted for diet 1 instead of 1.7 kg/d as observed. This unrealistic result would affect the behaviour of the BA model so much that a comparison amongst the models would become irrelevant. In the present study, appropriate parameter inputs were estimated. Nevertheless, it should be realized that the method of estimation might not correspond to the method the model-builders used. In particular, estimation of rumen particle dynamics is critical and complicates the interpretation of evaluation results.

Choosing parameter values that seem more appropriate than the published estimates caused the BA model to predict duodenal flow of NDF more accurately than the other models. However, other parameter estimates for rumen outflow might have improved NDF prediction in the DA and DY models also.

Neal *et al.* (1992) extensively evaluated the DY model and established, in contrast to the results of the present study, accurate predictions of duodenal flow of NDF. This contradiction might be explained by erroneous measurements of duodenal flow of NDF due to sampling errors and sample preparation. However, Van Vuuren *et al.* (1992) reasoned that these errors caused underestimation rather than overestimation of duodenal flows of NDF. Another possible explanation for the underprediction of duodenal NDF flow with the DY model might be a poor model performance with fractional outflow rates of OM as parameter input. The DY model was calibrated on an experiment in which Yb was used as a marker for particulate outflow that preferentially binds to small particles, whereas the total content of OM in the rumen as a marker includes large particles, small particles and soluble OM in rumen fluid. If the soluble fraction in rumen OM outflow is small compared with the particulate fraction (all NDF considered particulate matter and on roughage diets the majority of micro-organisms attached to particles), then the fractional outflow rate of total rumen OM pool is lower than that of the small particle pool. Thus, use of observed fractional outflow rates of rumen OM content in the present study might have resulted in an underestimated fractional outflow rate of small particles, an overpredicted retention and degradation of NDF in the rumen, and consequently an underpredicted duodenal flow of NDF. In the DA model,

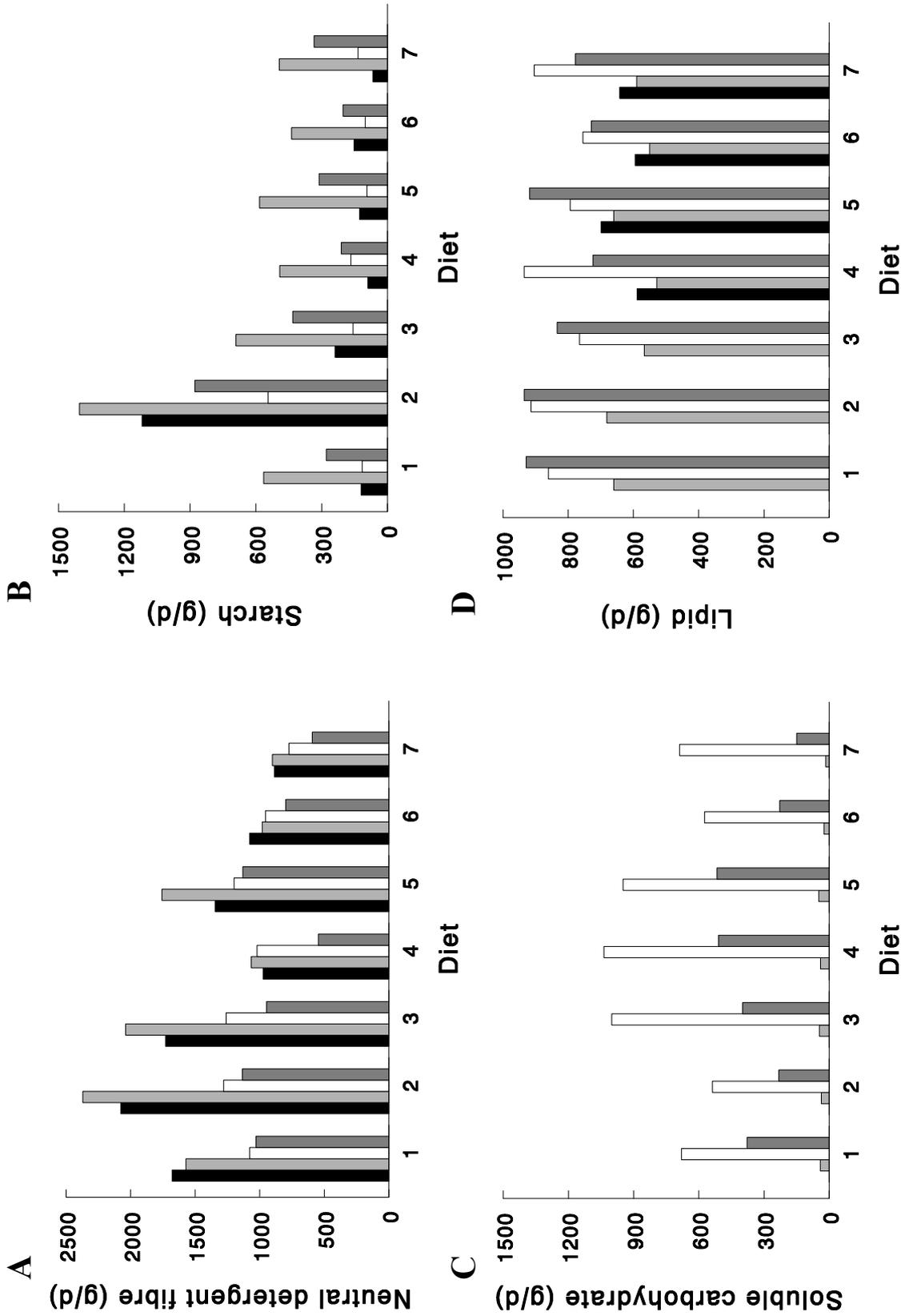


Figure 3. Comparison of observed (■) and predicted values of the duodenal flow of (A) neutral-detergent fibre, (B) starch, including solubilized starch, (C) soluble carbohydrate including solubilized NDF and excluding solubilized starch and (D) lipids on seven test diets by the models of Baldwin (1995; ■), Danfær (1990; □) and Dijkstra *et al.* (1992; ▒).

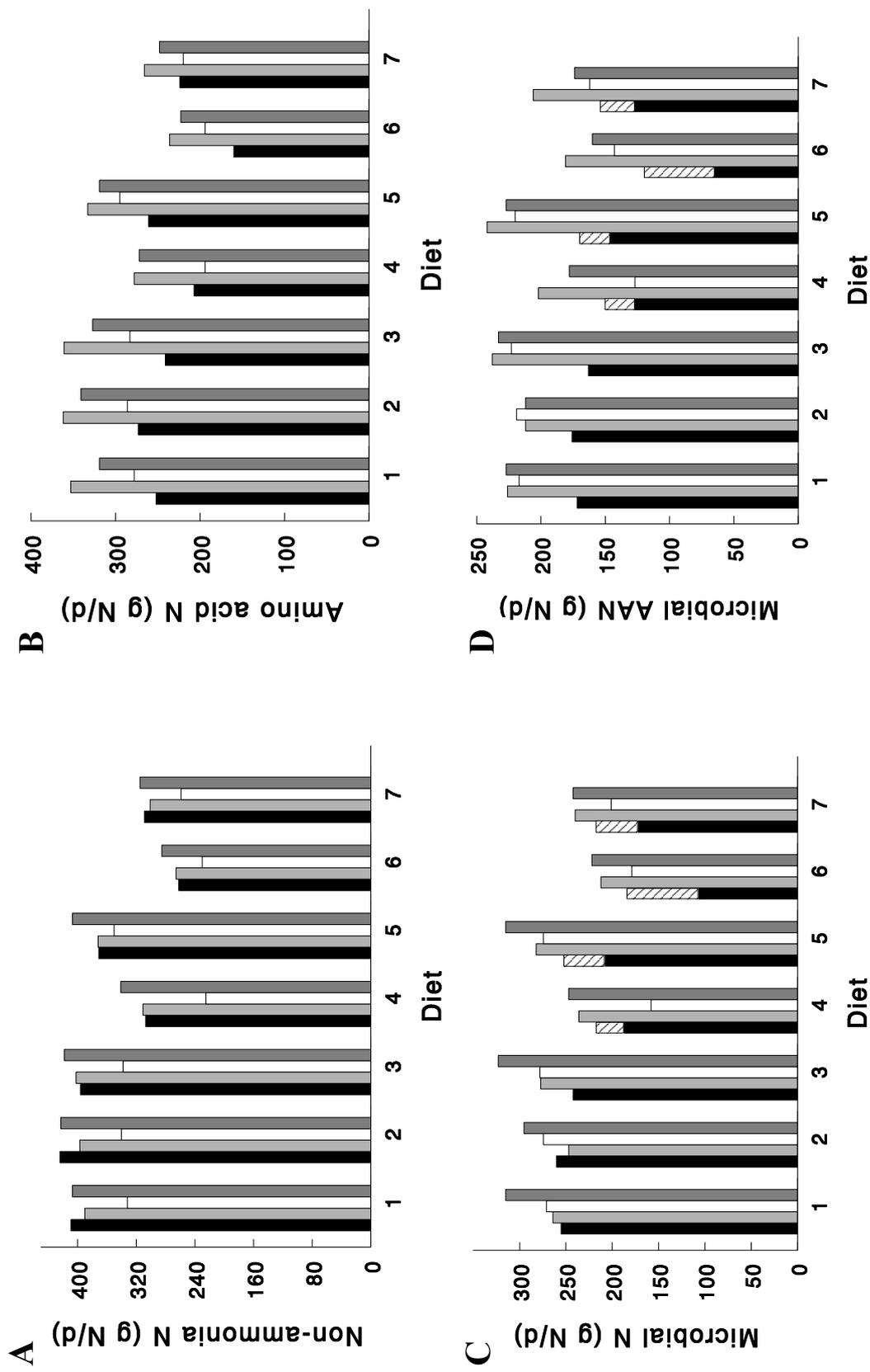


Figure 4. Comparison of observed (■) and predicted values of the duodenal flow of (A) non-ammonia N, (B) amino acid N, (C) microbial N and (D) microbial amino acid N on seven test diets by the models of Baldwin (1995; ■), Danfær (1990; □) and Dijkstra *et al.* (1992; ■). Observed microbial N fractions obtained with the amino acid profile method (■) and with the diaminopimelic acid (DAPA) method (▨) in diets 1-7.

predicted duodenal flow of NDF can additionally be manipulated by changing the value of the *G* parameter, but the same kind of argument might apply to this model. To conclude this section, the models identify areas of physical and chemical feed description necessary to represent specific relationships occurring in the rumen, but the present study indicates that these areas are still inadequately defined.

Nutrient flows

Large differences were established in the predicted transactions of carbohydrates and N in the rumen and in the resulting profile of nutrients that flow from the rumen to the duodenum (Tables 2 & 3; Figures 3 & 4). However, these results are susceptible to assumptions on the model inputs (already discussed for parameters in the previous section) and measurement errors in the experimental data used.

With the assumption that all feed non-AA NAN is immediately hydrolysed and constitutes an input to the rumen NH₃ pool, the predicted duodenal flow of non-AA NAN is assumed to be entirely of microbial origin and escaped feed N to be 100% AAN. However, the observed duodenal flows of non-AA NAN were much higher than the predicted values (Table 3), suggesting that some feed non-AA NAN did escape from the rumen. Considering rumen protein as crude protein ($\text{g N} \times 6.25$) instead of true protein (no NH₃ input from soluble N) will reduce predicted error of the duodenal flow of AAN and non-AA NAN. However, caution is necessary with explaining inaccurate prediction from the model representations because also the experimental estimates may be seriously biased.

Of all the measured duodenal flows, that of microbial matter probably has the largest standard deviation and is the least reliable. Therefore, not only the accuracy of model predictions but also the reliability of observed MN in particular should be evaluated. With the AA profile method, estimates of MAAN or MN were clearly lower with the AA profile method than with the DAPA method (Figures 4C & 4D). The percentage N of microbial origin was used to estimate the duodenal flow of MAAN and MN from the observed duodenal flow of AAN and NAN respectively, which seems to be valid at least on diets 4-7 because it resulted in ratios of duodenal flow of MAAN and MN that were comparable with that of the independently observed amounts of AAN and total N found in microbial matter isolated from duodenal samples (68% AAN). Variability of prediction errors was larger with the AA profile method and estimates of duodenal flow of MN and MAAN on diet 6 are so low that they are highly unlikely. The larger deviations between the predicted and observed duodenal flows of MN and MAAN with the AA profile method does not agree with the uniform prediction error of the duodenal flow of NAN or AAN for all diets (Figures 4A & 4B). From these arguments it may be concluded that the AA profile method in particular resulted in severely biased and inconsistent estimates of microbial mass in samples of the duodenal contents. In a direct comparison of alternative methods for the quantification of microbial matter, Siddons *et al.* (1982) also established much higher estimates of microbial N in rumen digesta of sheep with

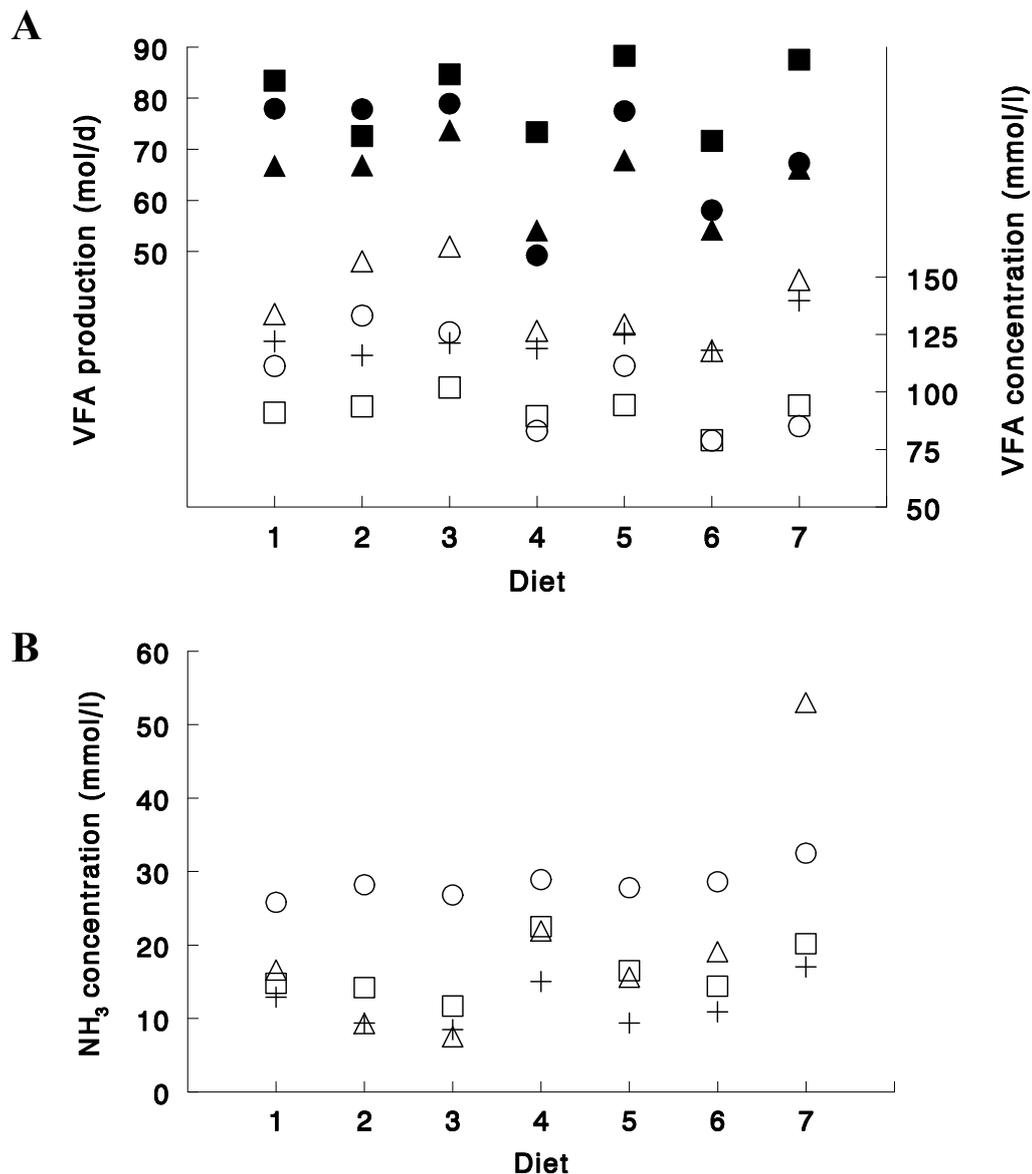


Figure 5. Comparison of observed (+) and predicted values of (A) volatile fatty acid (VFA) production (\square , \circ , \triangle) and concentration (\blacksquare , \bullet , \blacktriangle) and (B) ammonia concentration on seven test diets by the models of Baldwin (1995; \square), Danfær (1990; \circ) and Dijkstra *et al.* (1992; \triangle).

the DAPA method compared with the AA profile method. In contrast, Voigt *et al.* (1991) established low but comparable estimates with the AA profile and DAPA method in duodenal digesta of dairy cows. Thus, highly variable results have been obtained with different methods as well as within a single method of microbial quantification.

Further, protozoa were excluded with the isolation of microbial matter from digesta samples and also the DAPA method largely excludes protozoa (Broderick & Merchen, 1992). Thus, the presence of substantial amounts of protozoal N in duodenal MN might result in an underestimated experimental value of MN and consequently an overestimated duodenal flow

of feed N, feed AAN and non-AAN NAN. A more substantial contribution of protozoal N could be expected with diets 2 and 3 containing 330 g concentrates/kg DM intake (Dijkstra, 1994); however, there is no indication of a larger prediction error of MN flow (Figure 4C). An indication of an underestimated duodenal MN flow might be the much higher duodenal flows of non-AA NAN than can be explained by the outflow of microbial non-AA NAN (Table 3). In conclusion, there is a strong possibility of large bias in the experimental estimates of duodenal flow of MN and MAAN which seriously limits their use for model evaluation.

The models predicted different amounts of fermented substrate (Tables 2 and 3), accompanied by different VFA productions (Figure 5A). For predicting nutrient flows, the production of individual VFA is of particular interest because they are the largest energy fraction absorbed from the gastrointestinal tract (Reynolds *et al.*, 1994). However, no such observations were made in the experiments used. The only VFA observations available were those of concentrations in rumen fluid. The DY model uses the estimated VFA coefficients from Murphy *et al.* (1982) which describe the stoichiometry of the production of acetic, propionic, butyric and valeric acids with fermentation of soluble carbohydrate, starch, hemicellulose, cellulose and protein. These coefficient values resulted in a consistent underprediction of the molar percentages of acetic and butyric acids, and overprediction of those of propionic and valeric acids in rumen fluid. The BA model also uses these coefficient estimates, but with 1.0 mol propionic acid and 0.5 mol butyric acid substituted for 1.0 mol valeric acid. Further, coefficient estimates for protein fermentation were altered and VFA coefficients with fermentation of soluble carbohydrate and starch were made dependent on rumen pH (Argyle & Baldwin, 1988). These updated coefficient estimates resulted in the most accurate predictions of the molar percentages of individual VFA in rumen fluid. Earlier coefficient estimates of Baldwin *et al.* (1970) were applied in the DA model and resulted in the worst predictions. The different VFA absorption kinetics in the BA, DA and DY models will have contributed to these different results. The deviations found between predicted and observed molar percentages of individual VFA in rumen fluid are to be explained by incorrect prediction of the amount of fermented substrate, by incorrect coefficient values, or by incorrect absorption kinetics. Yet, it is uncertain to what extent these deviations may be considered representative of rumen VFA production.

Differences amongst models in the assumed microbial composition and substrate requirements for microbial synthesis can partially originate from the assumption in the BA and DA models that microbes incorporate rumen lipid, whereas the DY model ignores this. Besides the synthesis of the protein fraction in microbial mass, that of the lipid fraction might require the highest amount of substrate for generation of ATP or for incorporation into microbial mass (Czerkawski, 1986; Dijkstra *et al.*, 1992; Baldwin, 1995). The assumptions made in the BA, DA and DY models on the utilization of feed lipid will have influenced the parametrizations adopted for substrate requirement with microbial synthesis. Notwithstanding the small lipid content of the diets in the present study (31 to 41 g lipid/kg DM intake; Van

Vuuren *et al.*, 1992, 1993), such considerations are necessary to explain the higher carbohydrate incorporation in the DY model. Thus, different predictions of the amounts of carbohydrate, AAN and NH₃ that are incorporated and fermented with microbial synthesis must be explained from a different origin of the microbiological data used with model construction or from different assumptions on rumen conditions for microbial growth (Russell, 1984; Van Gylswyk & Schwartz, 1984; Czerkawski, 1986). To conclude this section on predicted nutrient flows, considerable differences exist in the predicted rumen transactions of carbohydrates and N. Although the differences between the BA, DA and DY models largely compensated for each other and resulted in more-or-less comparable predictions of the duodenal flows of OM, total carbohydrate, NAN and MN, large differences were established in the prediction of rumen transactions, duodenal flows of the individual carbohydrate and N fractions, and production rates of individual VFA.

Implications for future research

None of the models appeared to be clearly superior in predicting the nutrient flows to the duodenum. Calibration of the BA, DA and DY models apparently made them all predict the generally observed duodenal flows of OM, NAN and MN reasonably well. These comparable predictions result from a compensation of large and systematic differences that occurred in the simulated intra-ruminal flows. Forcing similar entry rates of soluble substrates into rumen fluid (according to the model descriptions the only substrate that can be utilized by micro-organisms) in the BA, DA and DY models was demonstrated to result in comparable differences in microbial metabolism to those found in the present study (Bannink & De Visser, 1995). Therefore, next to a different formulation of substrate availability, a different formulation of the microbial metabolism seems to cause the model differences established. Broadening the range of experimental conditions concerning amounts of feed intake, roughage:concentrate ratios, N content in feed, and types of roughage and concentrate will probably result in more distinct model behaviour (Dijkstra *et al.*, 1992; Neal *et al.*, 1992) and enable a more thorough evaluation of the models.

One aspect of rumen modelling that requires further research is resolving the representation of the microbial metabolism. The models tested differ strongly in concepts and theories applied because of the different objectives they were built with. The validity of these representations could not be tested with the observations used in the present study, although they appear to be the main distinction between the BA, DA and DY models and were shown to result in differing profiles of nutrient flows. More precise and detailed measurement of microbial and non-utilized feed fractions in carbohydrate, nitrogenous and other constituents in duodenal OM, might identify which representation of microbial metabolism is able to describe the transactions of substrates in the rumen. In this respect, the representation of microbial metabolism also needs to address the variation in the composition of microbial matter with changing dietary conditions (Danfær, 1990; Dijkstra *et al.*, 1992). Further, most

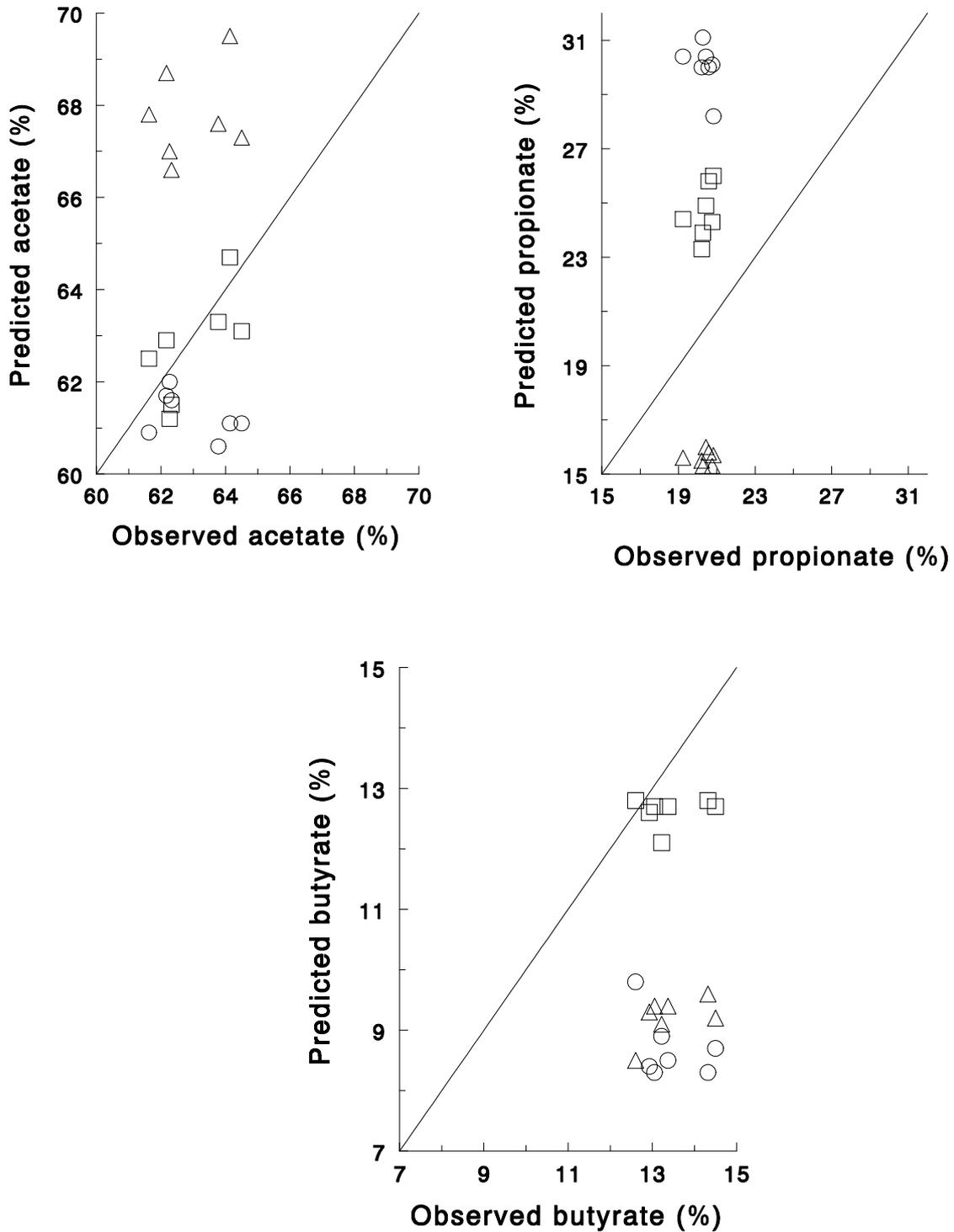


Figure 6. Comparison of observed and predicted values of (A) molar percentage of acetate, (B) molar percentage of propionate and (C) molar percentage of butyrate on seven test diets by the models of Baldwin (1995; □), Danfær (1990; ○) and Dijkstra *et al.* (1992; △). In the model of Dijkstra *et al.* (1992), the sum of predictions is less than 100 % because of additional valerate prediction.

ingested OM is fermented into VFA, but VFA production cannot be evaluated with observations of rumen concentrations. Rumen concentration of NH_3 additionally concerns MN synthesis from NH_3 and recycling of NH_3 to the rumen. The inconsistency between models in the present study indicates that further research is needed to represent these processes appropriately in rumen models.

Another aspect of rumen modelling that needs further consideration is the representation of particle dynamics and the interactions between particles, substrates and micro-organisms. Evaluation of the dynamic, instead of the steady-state, behaviour of the model might give further insight into the validity of concepts and theories used. Discrete meals administered in feeding trials cause large daily fluctuations of rumen contents and of nutrient flows from the rumen *in vivo* (Reynolds *et al.*, 1994). Despite the dynamic nature of the models, constant inputs are used with evaluations of simulated steady-state, whereas evaluations of dynamic simulations (Baldwin *et al.*, 1987; Danfær, 1990) have not yet been published (De Peters & Morris, 1984). Prediction of the daily fluctuation in rumen contents and duodenal flows will probably require inclusion of mechanisms that describe rumen particle dynamics and particle outflow (Sauvant & Ramangasoavina, 1991; Dijkstra *et al.*, 1992) in particular. However, introduction of more complex mechanisms in extant models might exacerbate a current weakness that is identified in this study: ambiguous or non-identifiable parameter inputs whose estimation can easily deviate from the method intended by the model-builders. For example, the BA model already includes particle kinetics described with a particle comminution rate and particle size factor. These parameter inputs might be estimated from observations of particle sizes of rumen contents, however, the meaning of these parameters also includes the more complex properties of substrate availability for microbial use which cannot be quantified easily (Baldwin *et al.*, 1987). The DA model has a further weakness in that it is not clear how to estimate the G parameter. If parameter inputs represent concepts that relate more closely to common rumen observations, models will become more easily falsified and model evaluation will give more decisive answers about the validity of the representations chosen.

In conclusion, this study identified large differences in the underlying mechanisms of microbial metabolism which were related to the different predictions of the profile of nutrient flows from the rumen. Extensive evaluations and comparisons on the same experimental data such as this work may challenge specific representations and enhance understanding of their consequences for predicted nutrient flows. It advances research into rumen function by illustrating the importance of understanding the mechanism of rumen microbial metabolism for accurate prediction of the individual nutrient flows from the rumen.

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References

- Argyle, J.L. & Baldwin, R.L., 1988. Modeling of the rumen water kinetics and effects on rumen pH changes. *Journal of Dairy Science* 71, 1178-1188.
- Baldwin, R.L., Lucas, H.L. & Cabrera, R., 1970. Energetic relationships in the formation and utilization of fermentation end-products. In: A.T. Phillipson, E.F. Annison, D.G. Armstrong, C.C. Balch, R.S. Cromline, R.S. Hardy, P.N. Hobson & R.D. Keynes (Eds.), *Physiology of Digestion and Metabolism in the Ruminant*, Oriel Press, Newcastle upon Tyne, United Kingdom, pp. 319-334
- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating cow. 11. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54, 107-131.
- Baldwin, R.L. (1995). *Modelling Ruminant Digestion and Metabolism*. Chapman & Hall, London, United Kingdom.
- Bannink, A. & De Visser, H., 1995. Comparison of mechanistic rumen models on microbial metabolism. In: D. Sauvant (Ed.), *Methods in Modelling Herbivore Nutrition. Satellite of Nth International Symposium on the Nutrition of Herbivores*, Institut National Agronomique Paris-Grignon and Institut National de la Recherche Agronomique, Paris, France.
- Bosch, M.W., 1991. *Influence of Stage of Maturity of Grass Silages on Digestion Processes in Dairy Cows*. PhD Thesis, Wageningen Agricultural University, The Netherlands.
- Broderick, G.A. & Merchen, N.R., 1992. Markers for quantifying microbial protein synthesis in the rumen. *Journal of Dairy Science* 75, 2618-2632.
- Czerkawski, J.W., 1986. *An Introduction to Rumen Studies*. Pergamon Press, Oxford, United Kingdom.
- Danfær, A., 1990. *A Dynamic Model of Nutrient Digestion and Metabolism in Lactating Dairy Cows*. PhD Thesis, Report 671, National Institute of Animal Science, Denmark.
- De Peters, E.J. & Moms, J.G., 1984. Discussion: rumen digestion and digestion end products. In: R.L. Baldwin & A.C. Bywater (Eds.), *Modeling Ruminant Digestion and Metabolism*. Proceedings of 2nd International Workshop, University of California, Davis, United States of America, pp. 63-68.
- Dijkstra, J., Neal, H.D.StC., Beever, D E. & France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *Journal of Nutrition* 122, 2239-2256.

- Dijkstra, J., 1994. Simulation of the dynamics of protozoa in the rumen. *British Journal of Nutrition* 72, 679- 699.
- Goswami, A.K. & Willcox, J.S., 1969. Effect of applying increasing levels of nitrogen to ryegrass. I. Composition of various nitrogenous fractions and free amino acids. *Journal of the Science of Food and Agriculture* 20, 592-595.
- McLeod, M.N. & Minson, D.J., 1988. Large particle breakdown by cattle eating ryegrass and alfalfa. *Journal of Animal Science* 66, 992-999.
- Murphy, M.R., Baldwin, R.L. & Koong, L.J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *Journal of Animal Science* 55, 411-421.
- Neal, H.D.StC., Dijkstra, J. & Gill, M., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model evaluation. *Journal of Nutrition* 122, 2257-2272.
- Nocek, J.E. & Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *Journal of Dairy Science* 74, 3598-3629.
- Poppi, D.P., Minson, D.J. & Ternouth, J.H., 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses. III. The retention time in the rumen of large feed particles. *Australian Journal of Agricultural Research* 32, 123-137.
- Reynolds, C.K., Harmon, D.L. & Cecava, M.J., 1994. Absorption and delivery of nutrients for milk protein synthesis by portal-drained viscera. *Journal of Dairy Science* 77, 2787-2808.
- Russell, J.B., 1984. Factors influencing competition and composition of the rumen bacterial flora. In: F.C.M. Gilchrist & R.I. Mackie (Eds.), *Proceedings of the International Symposium on Herbivore Nutrition in the Subtropics and Tropics*, The Science Press, Craighall, South Africa, pp. 313-345.
- Sauvant, D. & Ramangasoavina, B., 1991. Rumen modelling. In: J.-P. Jouany (Ed.), *Rumen Microbial Metabolism and Ruminant Digestion*, Institut National de la Recherche Agronomique, Paris, France, pp. 283-296.
- Siddons, R.C., Beever, D.E. & Nolan, J.V., 1982. Comparison of methods for the estimation of microbial nitrogen in duodenal digesta of sheep. *British Journal of Nutrition* 48, 377-389.
- Speckhart, F.H. & Green, W.L., 1976. *A Guide to Using CSMP - the Continuous System Modeling Program*. Prentice-Hall, New Jersey, United States of America.
- Van Gylswyk, N.O. & Schwartz, H.M., 1984. Microbial ecology of the rumen of animals fed high-fibre diets. In: F.C.M. Gilchrist & R.I. Mackie (Eds.), *Proceedings of the International Symposium on Herbivore Nutrition in the Subtropics and Tropics*, The Science Press, Craighall, South Africa, pp. 359-377.

- Van Vuuren, A.M., Krol-Kramer, F., van der Lee, R.A. & Corbijn, H., 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. *Journal of Dairy Science* 75, 2215-2225.
- Van Vuuren, A.M., van der Koelen, C.J. & Vroons-de Bruin, J., 1993. Effects of partial replacement of ryegrass by concentrates high in starch or fiber on protein digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science* 76, 2692-2700.
- Voigt, J., Schonhusen, U., Krawielitzki, R. & Piatkowski, B., 1991. Comparison of ¹⁵N, amino acid profile, RNA, DAPA and D-danine as markers for microbial nitrogen flowing to the duodenum of dairy cows. In: *Proceedings of the 6th International Symposium on Protein Metabolism and Nutrition. European Association of Animal Production Publication* no. 59, Herning, Denmark, EAAP, pp. 71-73.
- Woodford, S.T. & Murphy, M.R., 1988. Dietary alteration of particle breakdown and passage from the rumen in lactating dairy cattle. *Journal of Dairy Science* 71, 687-696.

Chapter 5

Causes of Inaccurate Prediction of Volatile Fatty Acids by Simulation Models of Rumen Function in Lactating Cows

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Causes of Inaccurate Prediction of Volatile Fatty Acids by Simulation Models of Rumen Function in Lactating Cows

Abstract

Extant mechanistic models of rumen function are unable to predict the molar proportion of volatile fatty acids (VFA) accurately. In order to make these models useful in investigating theories on nutrient flows that go beyond the rumen, the representations adopted need to be improved. This theoretical study was directed at identifying what parts of a rumen model may be responsible for the inaccurate VFA prediction. For five distinct parts of a model, not involving the description of the microbial metabolism, the hypothesis was tested that their inappropriate description can be a probable cause of inaccurate VFA prediction. These five parts were: (1) the input functions of feed ingestion; (2) the representation of insoluble, degradable substrate of roughage and concentrate origin; (3) the kinetics of VFA absorption from the rumen; (4) the VFA coefficients that describe the stoichiometry of the conversion of fermented substrate into VFA; and (5) the representation of the rumen particle dynamics. Every hypothesis was tested by modifying the model description and simulating it to steady state. Observations required were derived from digestion trials with lactating dairy cows. Simulation results demonstrated that the predicted molar proportion of rumen VFA concentrations is particularly influenced by VFA absorption kinetics and VFA coefficients. Although the description of particle dynamics also had a large influence with certain choices of its parameterization, it is probably a less important cause of inaccurate prediction when rumen feed degradation (apparent from rumen outflow) is predicted well. In conclusion, to obtain improved predictions of the molar proportions of rumen VFA, further work is required on the representation of VFA absorption kinetics and of VFA coefficients of fermentation stoichiometry.

Introduction

The physiological processes in the rumen have a decisive effect on the profile of nutrients entering the blood system after feed digestion. The majority of the ingested feed is converted into volatile fatty acids (VFA) by microbial fermentation of feed substrates and into microbial mass by microbial growth. The profile of nutrients absorbed from the gastrointestinal tract is an important concept in theories on the manipulation of ruminant production by the alteration of feeding (MacRae *et al.*, 1988; De Visser, 1993). Accurate prediction of absorbed nutrients is also necessary when feed characteristics are to be related to concepts and theories on intake regulation (Forbes, 1993; Gill & Romney, 1994), nutrient imbalances in animal metabolism (Poppi *et al.*, 1994) or eventual animal performance (De

Visser, 1993). To estimate the profile of absorbed nutrients with a certain diet requires a quantitative understanding of rumen function. Therefore, considerable work has been done on the integration of concepts and theories of rumen function in dynamic, deterministic simulation models. It is generally recognized that inaccurate simulation of the molar proportion of individual VFA by extant models (Dijkstra, 1994a) is one of the major limitations to further progress in prediction of nutrient absorption. The present paper addresses the concepts and theories used in modelling the dynamics of rumen VFA and the possible causes of its inaccurate prediction.

Five aspects of rumen function were identified from the literature that might be important for explaining deviation between predicted and observed rumen VFA. First, use of the rumen models to date has been almost totally restricted to simulations to steady state using constant inputs (Baldwin *et al.*, 19987; Neal *et al.*, 1992; Bannink *et al.*, 1996). However, in reality rumen inputs are discontinuous and consequently rumen contents are strongly fluctuating during the day (Vaage, 1992). Because rumen models are highly nonlinear, the integral of the simulated fluctuation of rumen VFA during the day with discontinuous inputs might be different from the simulated values with constant inputs (France *et al.*, 1982). Second, extant models adopt the concept of state variables that describe the rumen content of substrates of roughage as well as concentrate origin in a single pool. However, the differences in composition and in fractional degradation and rumen outflow rates of both types of feedstuff is the reason for the distinct names given to them. Therefore, pooling of roughage and concentrate feed is a major simplification and even more so when it is considered that roughages and concentrates are often fed separately. Third, the clearance of VFA from the rumen by absorption into the blood system is generally assumed to be similar for all VFA. But, there is some evidence on fractional absorption rates of individual VFA to differ in relation to factors including VFA concentration, pH and liquid volume (Dijkstra *et al.*, 1993). Fourth, inaccurate simulation of rumen VFA is generally attributed to the adopted values of VFA coefficients that describe the fermentation of distinct types of substrate into individual VFA. Coefficient values derived by Murphy *et al.* (1982) are thought to be the best available at the moment (Baldwin, 1995) and a refinement of this set of values has been published (Argyle & Baldwin, 1988). Fifth, the substrate availability for microbial use is represented differently in extant rumen models (Bannink & De Visser, 1997) which might have a different effect on the simulation of individual VFA. On the one hand, it is assumed that substrate pools are homogeneously distributed over the whole rumen volume. On the other hand, a heterogeneous distribution over rumen volume is assumed by adopting the concept of separate pools for specific particle sizes and description of the mechanism of their rumen dynamics (Baldwin, 1995). From these considerations, five components of rumen models were identified to be tested using simulation studies as a possible explanation of prediction errors of rumen VFA. The hypotheses are that simulated rumen VFA is affected by (1) discontinuous vs. constant inputs, (2) separated vs. pooled state variables for insoluble,

degradable substrate of forage and concentrate origin, (3) mechanism of particle dynamics versus a homogeneous distribution over the whole rumen volume.

Model of Rumen VFA Dynamics

A simple model of rumen function, containing a single substrate and single microbial species, is depicted in Figure 1 with the symbols defined in Table 1. Let $Q_d(t)$, $Q_s(t)$, $Q_m(t)$ and $Q_v(t)$ be state variables describing the quantity of insoluble, degradable substrate, soluble substrate, microbial matter and VFA in the rumen volume at time t , respectively, and their rate of change per unit time be

$$dQ_d/dt = \text{rumen input of } Q_d \text{ with feed intake} - \text{microbial degradation (hydrolysis) of } Q_d - \text{outflow of } Q_d \text{ from rumen} \quad [1a]$$

$$dQ_s/dt = \text{rumen input of } Q_s \text{ with feed intake} + \text{formation of } Q_s \text{ with substrate degradation} + \text{release of } Q_s \text{ with microbiological death of lysis and predation} - \text{utilization of } Q_s \text{ with microbial growth} - \text{outflow of } Q_s \text{ from rumen} - \text{absorption of } Q_s \text{ from rumen} \quad [1b]$$

$$dQ_m/dt = \text{growth of } Q_m \text{ on utilized soluble substrates} + \text{growth of } Q_m \text{ on predated other micro-organisms} - \text{death or lysis of } Q_m - \text{predation on } Q_m \text{ by other micro-organisms} - \text{outflow of } Q_m \text{ from rumen} \quad [1c]$$

$$dQ_v/dt = \text{rumen input of } Q_v \text{ with feed intake} + \text{synthesis of } Q_v \text{ with fermentation of soluble substrate or predated micro-organisms used for microbial growth} - \text{outflow of } Q_v \text{ from rumen} - \text{absorption of } Q_v \text{ from rumen} \quad [1d]$$

Several types of $Q_d(t)$, $Q_s(t)$, $Q_m(t)$ and $Q_v(t)$ have to be distinguished to describe the rumen function. The general description of the set of differential equations for a system with a total number of n state variables then becomes

$$dQ_d/dt = F_d(Q_1, \dots, Q_n) \quad d = 1, \dots, n_d \quad [2a]$$

$$dQ_s/dt = F_s(Q_1, \dots, Q_n) \quad s = n_d + 1, \dots, n_d + n_s \quad [2b]$$

$$dQ_m/dt = F_m(Q_1, \dots, Q_n) \quad m = n_d + n_s + 1, \dots, n_d + n_s + n_m \quad [2c]$$

$$dQ_v/dt = F_v(Q_1, \dots, Q_n) \quad v = n_d + n_s + n_m + 1, \dots, n_d + n_s + n_m + n_v \quad [2d]$$

where d , s , m and v indicate the type of state variable and n_d , n_s , n_m and n_v are the total number of state variables of type Q_d , Q_s , Q_m and Q_v , respectively, n is the sum of n_d , n_s , n_m and n_v , and F_d , F_s , F_m and F_v are functions of the Q_d , Q_s , Q_m and Q_v .

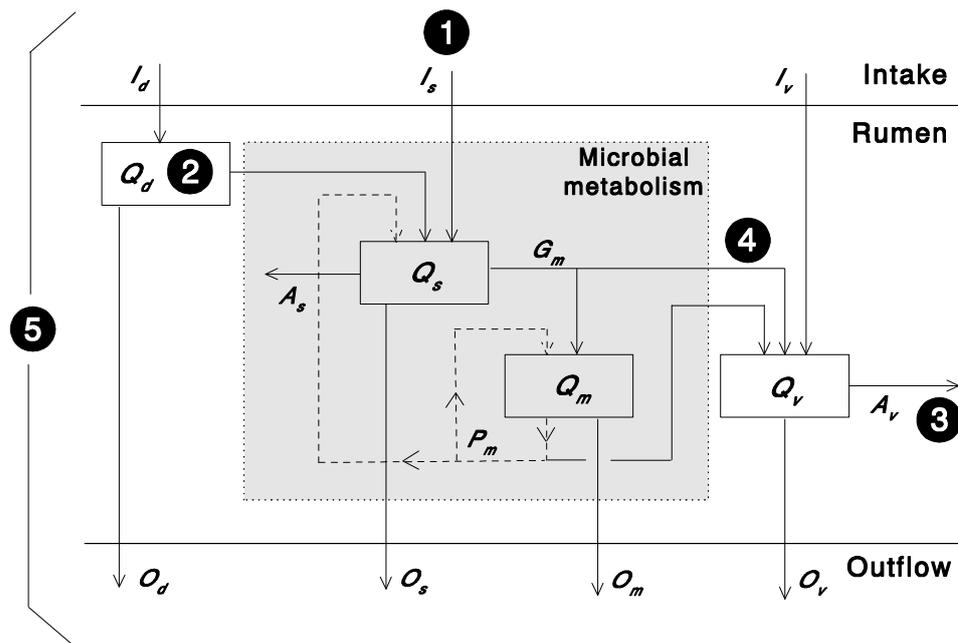


Figure 1. Simplified scheme of the nonlinear, deterministic models of rumen function with state variables Q_d , Q_s , Q_m and Q_v as rumen pools and functions A , G , I , L , O and P as fluxes to and from these pools. See text and Table 1 for precise explanation and definition. Encircled numbers indicate the parts of the model that were modified, dashed arrows indicate intra-ruminal recycling, whereas the dotted box indicates the description of the microbial metabolism.

Table 1. Symbols used in the description of the mechanistic rumen model

Quantity Q_x of rumen pool x (state variable)		Number n_x of distinguished rumen pools Q_x	
Q_d	insoluble, degradable substrate (mol)	n	total number of rumen pools
Q_m	microbial mass (g)	n_d	insoluble, degradable substrate pools
Q_s	soluble substrate (mol)	n_m	microbial pools
Q_v	volatile fatty acid (mol)	n_s	soluble substrate pools
		n_v	volatile fatty acid pools
Function $X(\dots)$ for flux to or from rumen pools (relationship between state variables)		Factor f_{Xy} for the fraction of flux $X(\dots)$ to Q_y	
A	absorption from rumen (mol/d)	f_{Gm}	fraction of substrate used for microbial growth that is incorporated into microbial mass
D	degradation of insoluble, degradable substrate (mol/d)	f_{Gv}	fraction of substrate used for microbial growth that is fermented into volatile fatty acids
G	microbial growth on soluble substrate (g/d)	f_{Pm}	fraction of predated microbial matter that is incorporated into the predated micro-organisms
I	rumen input with feed intake (mol/d)	f_{Pv}	fraction of predated microbial matter that is fermented into a specific volatile fatty acid
L	lysis or death of micro-organisms (g/d)		
O	outflow from rumen (mol/d or g/d)		
P	microbial predation (g/d)		

For a realistic description of rumen function the differential equations are essentially nonlinear. Because of this nonlinearity, analytical solution to these multiple substrate, multiple microbial species models of rumen function is limited if not impossible. Numerical techniques are used to solve the system of differential equations and to simulate model behaviour over time. With not too many recycling loops in the relationships between the Q_d , Q_s , Q_m and Q_v (e.g. lysis and predation of micro-organisms), the system can be expected to exhibit stable behaviour. A more explicit mathematical description of the multiple substrate, multiple microbial species model with I for rumen input with feed intake, D for degradation of insoluble substrate to a soluble form, G for microbial growth on soluble substrates, L for microbial lysis or death, P for predation of microbial matter by other micro-organisms, O for outflow from the rumen to the posterior gastrointestinal tract, and A for absorption from the rumen through the rumen wall per unit t is as follows

$$dQ_d/dt = I_d(t) - \sum_{d,m} D_d(Q_d, Q_m) - O_d(Q_d) \quad [3a]$$

$$dQ_s/dt = I_s(t) + \sum_{d,m} D_d(Q_d, Q_m) + \sum_{m,s} \{ L_m(Q_m, Q_s) - G_m(Q_m, Q_s) \} - O_s(Q_s) - A_s(Q_s) \quad [3b]$$

$$dQ_m/dt = \sum_{d,m,m^*} \{ G_m(Q_m, Q_s) \cdot f_{Gm} + P_m(Q_m, Q_s) \cdot f_{Pm} - L_m(Q_m, Q_s) - P_m(Q_m, Q_s) \} - O_m(Q_m) \quad [3c]$$

$$dQ_v/dt = I_v(t) + \sum_{m,m^*,s} \{ G_m(Q_s, Q_m) \cdot [1-f_{Gm}] \cdot f_{Gv} + P_m(Q_{m^*}, Q_s) \cdot [1-f_{Pm}] \cdot f_{Pv} \} - O_v(Q_v) - A_v(Q_v) \quad [3d]$$

where m^* will run as m in Equation [2c] but indicates a different type of microbe with $m^* \neq m$, f_{Gm} and f_{Gv} is the fraction of utilized soluble substrate Q_s that is incorporated into Q_m and fermented into Q_v , respectively, and f_{Pm} and f_{Pv} is the fraction of predated microbial matter Q_m that is incorporated into Q_m and the fraction of not-incorporated Q_m that is fermented into Q_v , respectively.

However, micro-organism m not necessarily uses every type of substrate s , only specific micro-organisms m predate on specific other micro-organisms m^* , some micro-organisms m are readily subject to lysis in the rumen whereas others are not, not every type of soluble substrate s is absorbed from the rumen, and, finally, degradation of an insoluble, degradable substrate d produces only one type of soluble substrate s . Thus, for a considerable number of d , s , m , m^* and v values the A , D , L , and P functions in Equation [3a] through [3d] are absent. The meaning of the symbols used is summarized in

The system described in Equation [3a] through [3d] is a hypothetical representation of rumen function. Alternative representations are possible, based on different concepts and theories, which results in different definitions of state variables and in other relationships between them. Dependent on the modelling goal, the choice can be made to represent certain aspects of rumen function in more or less detail. A number of these mathematical rumen models has already been constructed with different theories and concepts represented in them (see Bannink *et al.*, 1996, for a review). To simulate rumen function and rumen VFA dynamics in the present study, the model of Dijkstra (1994b) was selected because it considers important characteristics of rumen microbial metabolism that were not explicitly represented in other models. Of extant rumen models, this model gives the most detailed description of the rumen fermentation processes and VFA dynamics. Rumen volume and rates of rumen outflow are described by required parameter inputs. Because these inputs are observations of daily averages of rumen fluid content and fractional outflow rates of fluid and particulate matter (Dijkstra, 1994b; Bannink *et al.*, 1996), the objective of the model is to simulate fluxes per day. Like the functions for fluxes between rumen pools, some of the f factors (Table 1) that indicate a fraction of these fluxes in Equation [3a] through [3d] are nonlinear functions of the state variables. Further, the model distinguishes three Q_d state variables for insoluble, degradable starch (ST), cell wall carbohydrates (analysed as neutral detergent fibre or NDF) and protein (PRT); four Q_s state variables for soluble carbohydrate originating from dietary soluble carbohydrate (SC) or soluble ST, soluble carbohydrate originating from NDF, ammonia and soluble protein; five Q_m state variables for bacteria growing on SC and ST, bacteria growing on NDF, protozoa growing on bacteria and rumen substrate, the polysaccharide storage of the bacteria growing on SC and ST, and the polysaccharide storage of protozoa; and four Q_v state variables for the fermentation end-products acetic acid (AC), propionic acid (PR), butyric acid (BU) and valeric acid (VL).

Simulations and Data

Model simulations for testing hypothesis

Simulations with the model of Dijkstra (1994b) were performed in CSMP (Speckhart & Green, 1976) over 29 days using a fourth-order, variable step-length, Runge-Kutta integration method and a minimum and maximum step-length of 1.0×10^{-9} days and 2.5×10^{-3} days respectively, to ensure accurate integration with discontinuous inputs to the model. The unmodified model was used as published with simulation to steady state on constant inputs. Subsequently, the model was modified to test the five formulated hypotheses (summarized in Table 2):

(1) The effect was investigated of three different discontinuous inputs of I_d , I_s and I_v (part 1 in Figure 1; Tables 1 & 2) on simulation results; one pulse from 0.0 to 0.4 days; two pulses

with 60% and 40% of dry matter intake/day from 0.0 to 0.4 days and from 0.6 and 0.75 days, respectively; and four pulses with 40, 30, 20 and 10% of dry matter intake/d from 0.0 to 0.2 days, from 0.3 to 0.5 days, from 0.6 to 0.75 days and from 0.8 to 0.9 days, respectively.

(2) State variables and inputs of insoluble, degradable substrate were separated into those of roughage and concentrate origin, which doubled the number of Q_d state variables from 3 to 6 (part 2 in Figure 1; Tables 1 & 2). The mathematical description of the differential equations was kept identical to that of the corresponding pools in the unmodified model, except for the rumen input I_d . The value of f_{Gv} and of f_{Pv} is determined by the VFA coefficients and the fraction of utilized substrate that is fermented (Figure 1; Tables 1 & 2). For both the roughage and concentrate Q_d state variables, the same original set of VFA coefficients was used (Murphy *et al.*, 1982), and f_{Gv} and of f_{Pv} remained unaltered. Constant as well as observed intake patterns of concentrate and roughage (separate compositions and *in situ* degradation kinetics were distinguished for concentrate and roughage) were simulated with input functions I_d (parts 1 and 2 in Figure 1; Tables 1 & 2).

(3) The characteristics of VFA absorption kinetics A_v (part 3 in Figure 1; Tables 1 & 2) were altered by replacing the identical absorption equations with distinct ones for individual VFA that were derived from *in vivo* observations of AC, PR and BU in lactating dairy cows (Dijkstra *et al.*, 1993; similar equations taken for BU and VL). This modification to the model was tested both with a constant intake and with a division of the roughage and concentrate substrate and observed pulsed inputs of roughage and concentrate (parts 1-3 in Figure 1). In addition, simulations were performed with a constant fractional absorption rate (arbitrarily chosen to be equal to the parameter value of the maximum absorption rate of 7.86 mol/(l.d) Dijkstra, 1994b) that was identical for every individual VFA (A_v is a constant, part 3 in Figure 1; Tables 1 & 2), resulting in identical molar proportion of VFA concentration and production.

(4) The VFA coefficients in the model were replaced with alternative values of Argyle & Baldwin (1988), which changed the values of f_{Gv} and f_{Pv} (part 4 in Figure 1; Tables 1 & 2). Both the original and new set of values were derived from the estimates of Murphy *et al.* (1982), but in the new set production of PR and of BU was substituted for Murphy's VL production on the basis of 1 mol of PR and 0.5 mol of BU per mol of VL. The new coefficient values for SC and ST fermentation were functions of rumen pH.

(5) The concept of a homogeneous distribution of micro-organisms and insoluble substrates in the rumen represented by a single particle pool, was replaced with a mechanism for particle dynamics (Baldwin *et al.*, 1987) that distinguishes a large and a small particle pool in rumen contents. This mechanism describes the distribution of the Q_d and Q_m variables over a large and small particle pool, Q_{Lp} and Q_{Sp} , respectively, the flow of particles from the large to the small particle pool with a fractional comminution rate, $k_{Lp,Sp}$, and the partitioning of the dietary input over the large and small particle pool with the fraction of small particles in the diet, f_{Sp} (part 5 in Figure 1; Table 2). Then, the system of differential Equations ([3a] through

3d]) that describes the interactions between the Q_d , Q_s and Q_m state variables (Figure 1), only applied to their fractions present in the small particle and soluble pools of rumen contents. The incorporation of a mechanism of particle dynamics affects the value of the Q_d , Q_s and Q_m state variables in the rumen model, and consequently the simulated amount of substrate fermented into VFA. Three alternative parameterizations were simulated (Table 2) for reasons explained in previous work (Bannink *et al.*, 1996): (i) observed fractional outflow rates of rumen content of particulate matter as an estimate for the fractional outflow rate of small particles in the model, a fractional particle comminution rate of 4.5 /d, and 40% small particles in the diet; (ii) a similar simulation to (i) with observed fractional outflow rate of particulate matter, multiplied by the ratio of the simulated total particle content to small particle content of the rumen; (iii) a simulation similar to (ii) with the fractional comminution rate and fraction of small particles in the diet increased to 8.5 /d and 60%, respectively.

Experimental data

A considerable variation of model inputs was obtained with the selection of thirty-three experimental diets (diet 1 through diet 33; Tables 3 & 4) of seven feeding experiments with lactating dairy cows; Van Vuuren *et al.* (1993, diet 1 to 3), Van Vuuren *et al.* (1992, diet 4 to 7), Robinson *et al.* (1987, intake effect diet 8 to 12 and starch effect diet 13 to 17), De Visser *et al.* (1992, diet 18 to 21), De Visser *et al.* (1993, diet 22 to 25), De Visser *et al.* (1997, diet 26 to 29) and Klop & de Visser (1994, diet 30 to 33), respectively. All required inputs could be derived from the experimental data, except for the fractional outflow rate of rumen fluid in two experiments of De Visser *et al.* (1992, 1993) which was estimated with the regression equations of Owens & Goetsch (1986). In some experiments, fractional outflow rates were derived for several distinct particulate fractions in the rumen contents which may all serve as a measure of rumen particulate outflow. Because NDF is generally the largest contributor to VFA production, the value that appeared to result in the best prediction of rumen NDF outflow was applied in this study.

Observations of rumen VFA concentration (mmol of VFA/L; all diets) with the molar proportion of individual VFA (all diets) were used for evaluation of the model predictions. Observed molar proportion of AC, PR, BU and VL were calculated as the fraction in the total VFA observed (AC + PR + BU + VL) in rumen fluid (reported branched chain fatty acids including VL taken for VL with experiments of De Visser *et al.*, 1992, 1993).

Sensitivity and error of predicted VFA

Sensitivity of the modifications to the model was established by comparison of predicted amounts of substrate fermented into VFA, rumen VFA concentration, molar proportion of individual rumen VFA and rumen VFA production, with those of the unmodified model. Prediction errors were determined by comparing predicted VFA concentration and molar proportion of individual VFA with observed rumen VFA

concentrations. Prediction errors were calculated as the square root of the mean squared deviations between predicted and observed values, whereas changes by modification of the model were calculated as the square root of the mean squared deviations from predictions with the unmodified model. Both prediction errors and changes were expressed as percentage of the mean of the predictions with the unmodified model. Mean prediction errors or changes in simulation results with model modification were considered large when > 10%.

Table 2. Modifications to rumen model of Dijkstra (1994b) with constant inputs

Modified ¹ model part	Code	Description of modification ²
1	PI1	One pulse input functions I_d , I_s and I_v
1	PI2	Two pulses input functions I_d , I_s and I_v
1	PI3	Four pulses input functions I_d , I_s and I_v
2	RC1	Q_d state variables divided into substrate of roughage and concentrate origin, doubling n_d
1 + 2	RC2	Modification RC1, with observed patterns of pulsed inputs I_d , I_s and I_v of roughage and concentrate separately
3	AB1	Alternative absorption kinetics A_v of volatile fatty acids (Dijkstra <i>et al.</i> , 1993)
1 + 2 + 3	AB2	Combination of modification AB1 and RC2
3	AB3	Simplification of absorption kinetics A_v with an identical constant for all Q_v
4	VC	Alternative coefficients of volatile fatty acid production with substrate fermentation (Argyle & Baldwin, 1988), determining f_{Gv} and of f_{Pv}
5	PD1	Mechanism of particle dynamics included, its dynamics parameterized with $k_{Lp,Sp} = 4.5$; $f_{Sp} = 0.4$; $k_{o,Sp} = k_{o,Part}$ ³
5	PD2	As PD0 with parameterization replaced by $k_{Lp,Sp} = 4.5$; $f_{Sp} = 0.4$; $k_{o,Sp} = k_{o,Part} \times (Q_{Lp} + Q_{Sp})/Q_{Sp}$ ³
5	PD3	As PD1 with parameterization replaced by $k_{Lp,Sp} = 8.5$; $f_{Sp} = 0.6$; $k_{o,Sp} = k_{o,Part} \times (Q_{Lp} + Q_{Sp})/Q_{Sp}$ ³

¹ Numbering corresponding to that in Figure 1.

² Notation explained in text and in Table 1.

³ Notation: $k_{Lp,Sp}$ = fractional comminution rate of large particles to small particles (/d), f_{Sp} = fraction of small particles in feed, $k_{o,Sp}$ = fractional outflow rate of small particles (/d), $k_{o,Part}$ = observed fractional passage rate of particulate rumen content (/d), Q_{Lp} = quantity of large particles in rumen (kg), Q_{Sp} = quantity of small particles in rumen (kg).

Table 3. Conditions with thirty-three diets in seven feeding trials with lactating dairy cows that were used for the evaluation of model predictions.

Experiment	Description of diets	Dry matter ¹ intake (kg/d)	Concentrate content (%)	Milk	
				Production (kg/d)	Production (kg/d)
Van Vuuren <i>et al.</i> (1993)	<i>Fresh-cut ryegrass replaced with varying concentrate mixtures</i>				
	Diet 1: no replacement	16.3	10	24	38 ²
	Diet 2, 3: replacement with maize/hominy feed and sugar beet-pulp, resp.	16.3, 16.5	43, 44		
Van Vuuren <i>et al.</i> (1992)	<i>Fresh-cut ryegrass, varying in N fertilization and harvesting season</i>				
	Diet 4, 5: high and low N fertilization, resp., with summer harvest	13.3, 16.8	5, 7	26 ²	
	Diet 6, 7: low and high N fertilization, resp., with autumn harvest	13.0, 15.2	5, 6	14 ²	
Robinson <i>et al.</i> (1987)	<i>Ryegrass hay added with varying concentrate mixtures</i>				
	Diet 8-12: five decreasing levels of food intake with intermediate starch content (20%)	20.9, 17.0, 13.1	67 all	25.4, 19.5, 13.3	
	Diet 13-17: five increasing levels of starch content (from 8 to 32%) with intermediate intake level	9.2, 5.3 13.2, 13.1, 13.1	67 all	6.9, 0.0 13.5, 13.6, 12.8	
De Visser <i>et al.</i> (1992)	<i>Wilted ryegrass silage, maize silage and concentrate mixture, added with varying concentrates</i>	13.0, 13.0		13.0, 12.3	
	Diet 18, 19, 20, 21: barley, maize, pressed ensiled sugarbeet pulp, and moist ensiled maize bran added as concentrate, resp.	24.7, 24.4, 23.7	50 all	37.1, 38.2, 37.7	38.1
De Visser <i>et al.</i> (1993)	<i>Wilted ryegrass silage, with varying additions used during ensiling, and concentrate mixture</i>	24.3			
	Diet 22, 23, 24, 25: no addition, and molasses, formic acid and water added, resp.	21.9, 19.6, 20.0	56 all	35.9, 34.3, 34.4	35.8
De Visser <i>et al.</i> (1997)	<i>Fresh-cut ryegrass, varying in applied N fertilization rate and maturity</i>	21.0			
	Diet 26 & 27: low and high N fertilization, resp., early-cut	14.8, 15.6	12, 11	17.2, 18.5	
Klop & de Visser (1994)	Diet 28 & 29: low and high N fertilization, resp., late-cut	17.4, 14.9	10, 12	17.3, 17.3	
	<i>Concentrate mixture added with varying proportions of ryegrass silage and maize silage</i>				
	Diet 30, 31 & 32: grass silage decreasing from 45 to 25, to 5% of DM and maize silage increasing from 4 to 14, to 34% of DM, resp.	19.7, 21.2, 21.5	49 all	28.8, 28.7, 26.3	
	Diet 33: Diet 32 with 2 kg of starch pellets added	20.4	54	27.6	

¹ Reported values might differ from those used with simulations when measurement period of milk production did not coincide with measurement period of rumen fermentation or kinetics.

² Only average values were published.

Table 4. Variation in model inputs used in this simulation study

Diet ¹				Physical parameters ²			
	Mean	SD	Range		Mean	SD	Range
DMI (kg:d)	17.21	4.64	5.25 – 24.4	$k_{o,Liq}$ (/d)	2.90	0.69	1.55 – 4.51
F _c (%)	40.2	20.1	5 – 70	$k_{o,Part}$ (/d)	0.87	0.17	0.58 – 1.22
f _{CELL} (%)	48	5	36 – 55	Vol (L)	67.5	6.1	63.8 – 79.3
#meals ³	3.9	1.6	2 – 6				
Diet composition (g /kg DMI or g N /kg DMI)							
SC	99.2	51.7	18.0 – 218.1				
SST	52.2	40.6	0.8 – 135.0				
DST	39.1	33.5	3.0 – 140.0				
NDF	409.2	46.3	333.3 – 483.9				
DNDF	348.2	43.6	274.9 – 438.1				
N	28.1	2.9	19.2 – 35.1				
NH ₃ -N	0.8	0.9	0.0 – 3.0				
SN	9.3	4.0	3.2 – 16.8				
UN	2.6	1.1	1.1 – 5.0				
LI	6.4	3.9	29.1 – 41.7				

¹ Dietary inputs: DMI = Dry matter intake, DNDF = degradable NDF, DST = degradable starch, f_c = fraction concentrate in diet, f_{CELL} = fraction cellulose in NDF, LI = Dietary lipids, NDF = Neutral detergent fibre, NH₃-N = ammonia N, SC = soluble non-starch carbohydrate, SN = soluble N, SST = soluble starch, UN = undegradable N.

² Parameter inputs: $k_{o,Liq}$, $k_{o,Part}$ = fractional outflow rate of rumen liquid and particulate content, respectively, Vol = rumen liquid or non-dry matter content.

³ Number of separate administrations of roughage and concentrate meals (including concentrate supplements), or of mixed meals.

Simulation Results

Effect on predicted amounts of fermented substrate

The deviation between predicted amounts of fermented substrate with the unmodified and modified model are presented in Table 5. Modification of VFA absorption kinetics or the values of the VFA coefficients did not influence substrate fermentation, resulting from the mathematical formulation of the fermentation mechanism. Predictions of the fermented amount of NDF and PRT were most altered with the inclusion of the mechanism for particle dynamics, whereas discontinuous inputs most affected fermented amounts of SC. In addition to these effects of the modifications, fermented amounts of ST also changed with the division of insoluble, degradable substrate into a roughage and concentrate pool. The effects of the division of substrate and the application of pulsed inputs were additive when the combination

of the two was tested. Only inclusion of the mechanism for particle dynamics caused a large alteration of the fermented amount of organic matter (OM) (as the sum of fermented SC, ST, NDF and PRT).

Table 5. Deviation between predicted amounts of fermented substrate with the unmodified and modified model of Dijkstra (1994b)

	FSC	FST	FNDF	FP	FOM ¹
Mean predicted value with unmodified model	936	791	3064	1436	6437
<i>Simulation</i> ²	<i>Deviation (% of mean predicted value with unmodified model)</i>				
PI1	37.7	17.6	6.2	19.5	6.2
PI2	29.2	22.4	4.4	10.6	2.8
PI3	25.3	21.9	2.4	4.3	1.3
RC1	9.4	19.9	3.8	2.6	2.5
RC2	38.5	29.7	5.6	13.3	4.2
AB1	0.0	0.0	0.0	0.0	0.0
AB2	38.5	29.7	5.6	13.3	4.2
AB3	0.0	0.0	0.0	0.0	0.0
VC	1.0	0.9	2.3	2.1	0.9
PD1	9.4	17.0	13.1	51.0	17.7
PD2	9.2	15.5	54.1	45.8	36.2
PD3	9.3	11.9	21.0	21.2	14.7

¹ FSC = fermented soluble carbohydrate, FST = fermented starch, FNDF = fermented neutral detergent fibre, FP = fermented protein, FOM = fermented organic matter.

² Explanation of codes for simulations in Table 2.

Effects on predicted rumen VFA

The changes in predicted VFA production (Table 6) were most pronounced with the alternative set of VFA coefficients and with the incorporation of the mechanism for particle dynamics. The magnitude of change was dependent on the parameterization chosen and resulted from changes in amount of fermented OM (Table 5). The VFA concentration changed with every modification except with the different patterns of pulsed inputs (Table 6). Predicted molar proportions of individual VFA were strongly affected by the alternative absorption kinetics and alternative VFA coefficients, whereas the different patterns of pulsed inputs had small effects (Table 6). The division of insoluble, degradable substrate into roughage and concentrate pools affected the predicted molar proportions of PR and VL to a

higher extent than that of AC and BU (Table 6), whereas its combination with observed pulsed inputs increased the change of predicted molar proportion of BU. Inclusion of the mechanism for particle dynamics had small or large effects on predicted molar proportions of VFA, dependent on the parameterization chosen.

Prediction accuracy of un modified model

Although changes in predicted VFA concentration and production with different diets resembled observed changes in rumen VFA concentrations for most diets (data not shown), the prediction error of VFA concentration was large (Table 7). Deviations between observed and predicted molar proportions of AC, PR, BU and VL were clearly dependent on the experi-

Table 6. Deviation between predicted rumen volatile fatty acids with the unmodified and modified model of Dijkstra (1994b)

	cVFA	pVFA ¹	fAc	fPr	fBu	fVI
Mean predicted value with unmodified model	(mmol/l) 148.8	(mol/d) 68.3	0.642	Molar proportion 0.192 0.083 0.083		
<i>Simulation</i> ¹	<i>Deviation (% of mean predicted value with unmodified model)</i>					
PI1	4.4	5.8	3.2	5.1	8.1	9.8
PI2	2.5	2.6	1.0	1.8	4.8	4.6
PI3	1.3	1.3	0.5	1.2	3.6	2.7
RC1	15.2	2.6	4.3	11.7	2.8	8.1
RC2	12.2	4.0	4.5	12.8	8.6	9.3
AB1	16.4	0.0	23.1	44.6	37.4	39.0
AB2	16.7	3.9	26.0	51.2	40.9	43.7
AB3	-- ³	0.0	4.1	6.0	8.8	9.5
VC	12.6	11.0	18.5	41.7	47.9	-- ⁴
PD1	15.9	15.5	1.8	2.6	3.0	19.2
PD2	35.3	34.8	5.4	13.7	20.4	11.6
PD3	14.1	14.0	1.7	4.3	6.2	3.0

¹ Abbreviations: fAc, fPr, fBu, fVI = molar proportion of rumen acetic acid, propionic acid, butyric acid, and valeric acid, respectively, in total rumen volatile fatty acids; cVFA = concentration of volatile fatty acids, pVFA = production of volatile fatty acids.

² Explanation of codes for simulations in Table 2.

³ Arbitrary choice of absorption constant makes predicted cVFA irrelevant.

⁴ Volatile fatty acid coefficients of Argyle & Baldwin (1988) do not consider valeric acid production.

ment (Figure 2). The proportion of AC (Figure 2A) was overpredicted with diet 1 to 7 and diet 18 to 33, in contrast to underprediction with diet 8 to 17 from the experiment of Robinson *et al.* (1987). A reversed pattern was obtained for the deviation of the predicted proportion of PR (Figure 2B). The proportion of BU and VL was highly underpredicted (Figure 2C) and overpredicted (Figure 2D), respectively, with all diets. Percentage of mean prediction error with the unmodified model was large and increased from AC, PR, BU to VL (Table 7) corresponding to decreased amounts present in rumen fluid.

Table 7. Prediction errors of rumen volatile fatty acids with the unmodified and the modified model

	fAc ¹	fPr	fBu	fVl	cVFA
	Molar proportion				(mmol/l)
Observed ²	0.636 (0.023) ³	0.204 (0.024)	0.141 (0.014)	0.019 (0.007)	121.4 (14.2)
Predicted ⁴	0.642 (0.037)	0.192 (0.029)	0.083 (0.011)	0.083 (0.013)	148.8 (41.8)
Simulated ⁵	<i>Prediction error (% of mean predicted value with unmodified model)</i>				
Unmodified	5.4	19.4	71.5	78.1	30.5
PI1	6.2	21.7	76.4	72.3	30.0
PI2	5.3	20.5	73.1	78.4	29.3
PI3	5.2	20.3	72.3	79.2	30.0
RC1	6.7	24.0	71.9	75.5	43.4
RC2	6.7	25.6	74.8	75.4	40.0
AB1	24.8	54.3	106.9	39.3	25.9
AB2	27.1	59.2	111.3	38.0	24.0
AB3	5.9	18.0	63.3	87.0	-- ⁶
VC	10.8	50.6	31.0	-- ⁷	41.4
PD1	4.6	20.3	73.5	95.0	42.0
PD2	8.6	22.1	63.2	72.3	27.2
PD3	5.9	19.2	69.1	77.1	26.5

¹ fAc, fPr, fBu, fVl = molar proportion of rumen acetic acid, propionic acid, butyric acid, valeric acid, respectively, in total rumen volatile fatty acids; cVFA = concentration of volatile fatty acids.

² Average of observed values.

³ Standard deviation.

⁴ Average of predicted value with unmodified model.

⁵ Explanation of codes for simulations in Table 2.

⁶ Arbitrary choice of absorption constant makes predicted cVFA irrelevant.

⁷ Volatile fatty acid coefficients of Argyle & Baldwin (1988) do not consider valeric acid production.

Effects on prediction accuracy

None of the modifications improved the prediction accuracy of the molar proportion of AC, PR, BU or VL (Table 7). Although the accuracy of BU improved with the alternative VFA coefficients, prediction errors of AC and PR became much larger. This was also the case for the improved prediction of the VL fraction with the alternative absorption kinetics. Because the prediction error of VFA concentration is largely determined by the overprediction with diet 18 to 25, a general decrease in predicted values with the alternative absorption kinetics slightly reduced this error. Prediction errors of VFA concentration remained large however (Table 7).

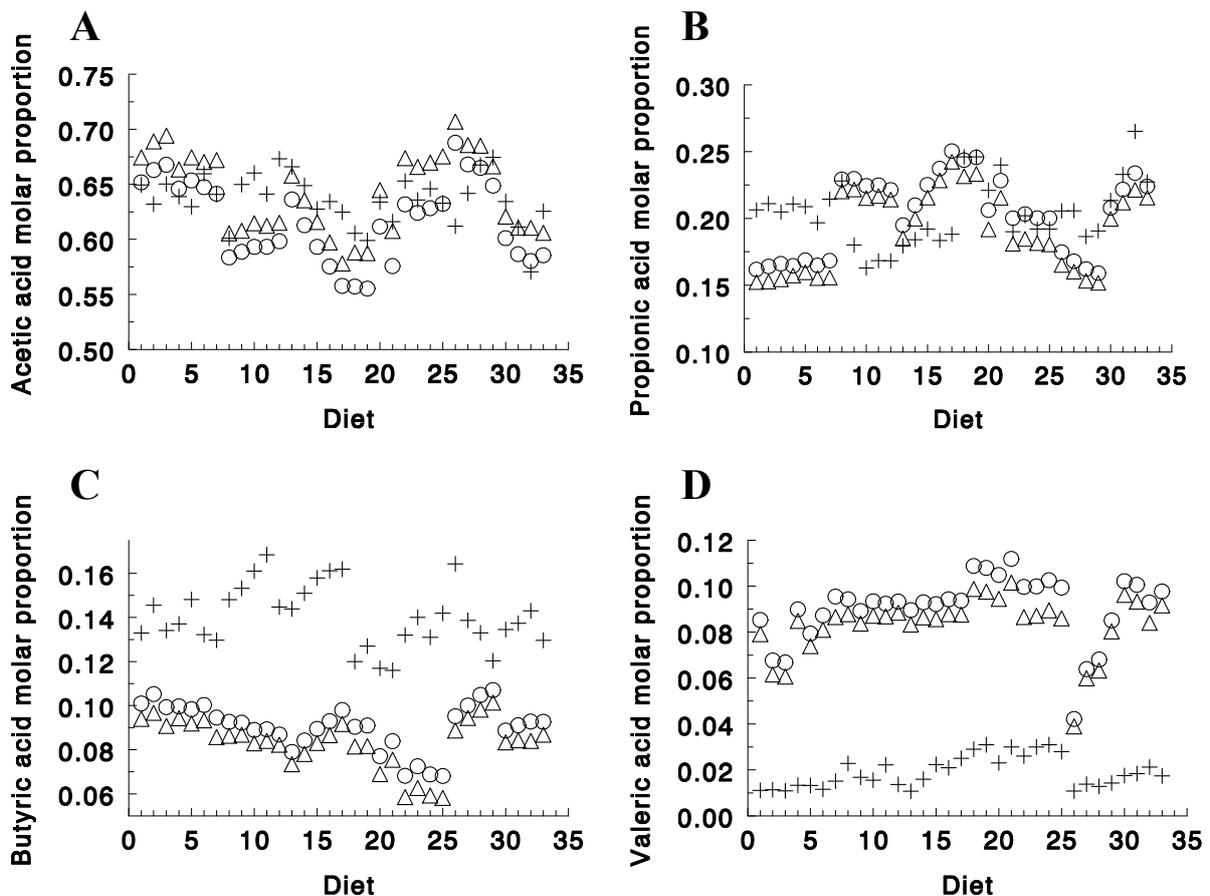


Figure 2. Comparison between observed (+) and predicted (△, concentration; ○, production) molar proportion of acetic acid (A), propionic acid (B), butyric acid (C) and valeric acid (D) in rumen fluid.

Discussion

The modified parts of the rumen model did not include alterations to the representation of microbial metabolism (Figure 1). Therefore, to conclude from the simulation results what parts must be altered to improve VFA prediction, the implicit assumption must be made that

microbial metabolism is described correctly and predicts the amounts of SC, ST, NDF and PRT fermented into VFA accurately. It can be reasoned that prediction errors to the molar proportion of rumen VFA concentrations are unlikely to be caused by erroneous predictions of the microbial activity when the amounts of rumen outflow of OM, non-ammonia N and microbial N (and thus their apparent rumen degradability) are predicted well (Neal *et al.*, 1992). Although this reasoning seems justified, it can be incorrect when separate flows in the rumen are predicted erroneously, but their errors compensate and still result in reasonable predictions of rumen outflow (Bannink & De Visser, 1997). No observations were available to evaluate the simulation results of intra-ruminal flows. Besides this uncertainty, the correctness of the simulated partitioning of the microbial utilization of individual substrates for fermentation into VFA and incorporation into microbial mass (Figure 1) must also implicitly be assumed. This assumption is thought to be reasonable because the model applies a variable partitioning of utilized substrate with variation of microbial growth rate; an increased fraction of utilized substrates fermented into VFA with a decreased microbial growth rate, which is in line with observed growth characteristics of rumen micro-organisms (Dijkstra, 1994b, Russell & Wallace, 1988). Throughout the rest of this discussion, it is assumed that an inaccurate description of the intra-ruminal flows and of the partitioning of utilized substrates over VFA and microbial mass contributed to a small extent to the prediction errors of rumen VFA, compared to the contribution of inaccurate description of other processes than microbial growth.

Implications of simulation results

In the present study, the modifications affected almost every part of the model, apart from the microbial metabolism (Figure 1). The modifications can be distinguished into two types. On the one hand, discontinuous inputs, separation of roughage and concentrate pools of degradable substrate, and an alternative representation of rumen particle dynamics only affect simulated rumen VFA by alteration of the amounts of degraded OM per day. On the other hand, alternative VFA absorption kinetics and alternative VFA coefficients only affect the state variables of rumen VFA and thereby the daily average of the concentration and molar proportion of rumen VFA. According to the mathematical formulation of the model, the first type of modification affects rumen pool sizes of the interacting state variables of substrate and micro-organisms, and thereby the amounts of fermented substrate (Table 5). However, the effect of these modifications on predicted concentration, molar proportion and production of rumen VFA remained small, except for the included mechanism for particle dynamics (Table 6). For the second type of modification, the mathematical formulation of the model only allows changes in simulated rumen VFA (not in amounts of fermented substrate), which appeared to be significant in the present study (Table 6). Although these large effects on VFA prediction did not decrease the prediction errors (Table 7), it does indicate that in particular an

improved description of VFA absorption and VFA coefficients may contribute to an improved prediction of molar proportion of rumen VFA concentrations.

Inclusion of the alternative representation of rumen particle dynamics caused large alterations in simulated substrate degradation and fermentation into VFA. However, if the unmodified model already predicts the amount of rumen outflow of distinct digesta fractions per day accurately, there is no need to modify the representation of particle dynamics for improved predictions of daily fluxes. In the present model, the representation of particle dynamics was fully determined by the parameter inputs of the daily average of rumen volume (fluid content) and of the fractional outflow rate of fluid and particulate matter. A reasonable prediction of NDF outflow per day was ensured in the present study, because its accuracy is most decisive for accurate simulation of rumen function. Consequently, the contribution of an inaccurate prediction of particle dynamics or substrate degradation to the established prediction errors of VFA is thought to be much smaller than that of erroneous VFA absorption kinetics and VFA coefficients.

The considerations above leave two parts of the present model that are most likely involved in the incorrect prediction of the molar proportion of rumen VFA; the absorption kinetics of individual VFA and the VFA coefficients. Incorrect VFA coefficients are a very plausible cause of the prediction errors for a number of reasons. Firstly, the values of the coefficients were fitted with a model that assumed identical absorption rates of the individual VFA and a fixed microbial growth efficiency for all diets and all amounts of feed intake (Murphy *et al.*, 1982). However, these assumptions are probably incorrect in most cases. Fractional absorption rates of individual VFA might be different *in vivo* (Dijkstra *et al.*, 1993), but particularly the efficiency of microbial growth is known to be variable because of the influence of factors including the fractional growth rate of the micro-organisms, the availability of preformed substrates for this growth, the energy utilization by micro-organisms for non-growth functions, the composition of the micro-organisms and the turnover of microbial matter in the rumen (Russell & Wallace, 1988). Another important argument against the appropriateness of the VFA coefficients is that the data used with their estimation are probably not representative of lactating cows. Most of the data were obtained from experiments on sheep or steers, with different rumen characteristics and diets, and amounts of daily feed intake that are much closer to maintenance level. It appeared that the range of observed molar proportions of VFA used in the present study (Figure 2) was smaller than that used for coefficient estimation by Murphy *et al.* (1982). Although the principles of the ecology of rumen micro-organisms might be similar in different species of ruminants or in different fermentation conditions (Van Soest, 1994), the quantities found of distinct types of micro-organisms differ (Dehority & Orpin, 1988). This is also illustrated by a separate set of VFA coefficients derived for roughage and concentrate diets (Murphy *et al.*, 1982). The term concentrate is used for high-quality, low-fibre feeds which contain a high concentration of digestible energy per unit weight and volume, whereas roughage is used for pastures, crops,

legumes, and so on, which are characterized by a larger cell wall fraction and a larger volume per unit of weight (Van Soest, 1994). Hence, strongly differing diets, like diets predominantly composed of roughages as opposed to those composed of concentrates, or like diets fed in tropical regions as opposed to those fed in temperate regions, will result in different rumen conditions and different fermentation stoichiometry. Nevertheless, specific modifications to a basic conceptual model, like the one used in the present study, might serve for the representation of each of these rumen conditions. A focus on rumen conditions that are specific for The Netherlands, does not make the simulation results irrelevant for other conditions. On the contrary, by leaving out conditions which differ strongly the conclusions could be drawn more justifiably. Moreover, the present investigation is mainly of a theoretical nature and hence the results can be regarded to demonstrate model features rather than dietary features. Finally, other factors not considered with the estimation of the current set of VFA coefficients might be necessary to explain the observed molar proportions of VFA, like rumen pH (Argyle & Baldwin, 1988), microbial growth rate, type of microbe fermenting and type of substrate fermented, and predation of micro-organism (Dijkstra, 1994a). There is thus some doubt about the applicability of current values of VFA coefficients. However, the observed molar proportion of rumen concentrations of individual VFA is the apparent outcome of the integrated contributions of all factors that determine this molar proportion (Dijkstra, 1994a). Therefore, derivation of relationships for the contribution of these factors *in vivo*, independently from each other, is severely hampered with VFA concentrations as the only rumen observations available.

Improvement of VFA prediction

A possible approach to improve the description of molar VFA proportions in rumen models, is to use more representative measurements of rumen VFA concentrations. By selection of data from rumen digestion trials with lactating cows, probably more appropriate VFA coefficients can be estimated for this type of animal in terms of the concepts and theories applied in the model. It should be noted, however, that the assumptions made on the absorption kinetics of the individual VFA will have a profound influence on the estimated values. Moreover, such an estimation exercise should preferably be undertaken with a description of rumen fermentation processes that has less drawbacks than the one that was used to fit current VFA coefficients by Murphy *et al.* (1982). The description to be used for estimation of VFA coefficients should be capable to explain the observed variation in microbial growth efficiency with dietary changes, like for example the model used in the present study.

Evaluation of predicted rates of VFA production is to be preferred above evaluation on predicted rumen VFA concentrations, when the aim is to use the model for investigating theories on nutrient flows. Hence, accurate measurements of VFA production rates would allow a more decisive evaluation of current VFA coefficients. However, such data are not

available for lactating cows and evaluation of VFA prediction is thus necessarily restricted to rumen concentrations that are routinely measured. An alternative approach to improve the predicted molar proportion of VFA produced, is evaluation with measurements of the net amount of VFA that is absorbed from the gastrointestinal tract and appears in portal blood. Although more such nutrient flux measurements are being performed, they do not fall within the scope of the rumen model. Firstly, these portal fluxes quantify the amount and molar proportion of VFA absorbed from the entire gastrointestinal tract, including the VFA produced in the gut post the rumen. Secondly, with absorption, part of the VFA is metabolized by tissues in the wall of the gastrointestinal tract (Bergman, 1990). Consequently, observed net fluxes of VFA in portal blood do not coincide with the VFA production in the rumen and thus can only give a qualitative indication of prediction accuracy of a rumen model.

Summarizing, the issue of the molar proportion of individual VFA found or, more preferably, produced within the rumen, needs to be addressed in future rumen modelling and probably requires an improved description of the values of VFA coefficients and the factors influencing these values. If observed net fluxes of VFA in portal blood are to be used for evaluation and to improve VFA predictions, the mechanism of VFA absorption and the metabolism of VFA during their absorption needs to be represented.

Conclusions

As predicted by the extant model, nutrient flows from the rumen are too inaccurate to use the model for investigating theories that go beyond the gastrointestinal tract. Further development of the rumen model particularly needs to address the improvement of the rate and the molar proportion of VFA production. Prediction errors of the molar proportion of rumen VFA concentrations are most likely caused by erroneous description of the VFA absorption kinetics and the VFA coefficients, and, possibly, other factors have to be included to explain the observed variation in rumen VFA concentrations. Testing of the effects of pulsed inputs, division into degradable roughage and concentrate substrate pools, and inclusion of a mechanism for particle dynamics demonstrated an influence on model behaviour. Although these aspects may be expected to be particularly important for description of the physical factors regulating rumen function (rumen fill, distribution of particle size, rumen outflow rates; Kennedy & Murphy, 1988), they seem to be less important in explaining prediction errors of the molar proportion of VFA found in the rumen, provided the model represents the microbial metabolism well, and substrate degradation and partitioning of utilized substrate over microbial mass and VFA was predicted reasonably in the present study.

References

- Argyle, J.L. & Baldwin, R.L., 1988. Modelling of rumen water kinetics and effects of rumen pH changes. *Journal of Dairy Science* 71, 1178-1188.
- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *Journal of Dairy Science* 54, 107-131.
- Baldwin, R.L., 1995. *Modeling Ruminant Digestion and Metabolism*. Chapman & Hall, London, United Kingdom.
- Bannink, A., De Visser, H., Dijkstra, J. & France, J., 1996. Impact of diet specific input parameters on simulated rumen function. *Journal of Theoretical Biology* 184, 371-384.
- Bannink, A. & De Visser, H., 1997. Comparison of mechanistic rumen models on mathematical formulation of extramicrobial and microbial processes. *Journal of Dairy Science* 80, 1296-1314.
- Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70, 567-590.
- Dehority, B.A. & Orpin, C.G., 1988. Development of, and natural fluctuations in, rumen microbial populations. In: P.N. Hobson (Ed.), *The Rumen Microbial Ecosystem*, Elsevier Science Publishers, London, United Kingdom, pp. 151-183.
- De Visser, H., Van der Togt, P.L., Huisert, H. & Tamminga, S., 1992. Structural and non-structural carbohydrates in concentrate supplements of silage-based dairy cow rations. 2. Rumen degradation, fermentation and kinetics. *Netherlands Journal of Agricultural Science* 40, 431-445.
- De Visser, H., Huisert, H., Klop, A. & Ketelaar, R.S., 1993. Autumn-cut grass silage as roughage component in dairy cow rations. 2. Rumen degradation, fermentation and kinetics. *Netherlands Journal of Agricultural Science* 41, 221-234.
- De Visser, H., 1993. *Influence of Carbohydrates on feed intake, rumen fermentation and milk performance in high-yielding dairy cows*. PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- De Visser, H., Valk, H. & Klop, A., 1997. Nutrient fluxes in splanchnic tissue in dairy cows: Influence of grass quality. *Journal of Dairy Science*, 80, 1666-1673.
- Dijkstra, J., Neal, H.D.StC., Beever, D.E. & France, J., 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *British Journal of Nutrition* 69, 385-396.
- Dijkstra, J., 1994a. Production and absorption of volatile fatty acids in the rumen. *Livestock Production Science* 39, 61-69.
- Dijkstra, J., 1994b. Simulation of the dynamics of protozoa in the rumen. *British Journal of Nutrition* 72, 679-699.
- France, J., Thornley, J.H.M. & Beever, D.E., 1982. A mathematical model of the rumen. *Journal of Agricultural Science (Cambr.)* 99, 343-353.

- Forbes, J.M., 1993. Voluntary feed intake. In: J.M. Forbes & J. France (Eds.), *Quantitative Aspects of Ruminant Digestion and Metabolism*, CAB International, Wallingford, United Kingdom, pp. 479-494.
- Gill, M. & Romney, D., 1994. The relationship between the control of meal size and the control of daily intake in ruminants. *Livestock Production Science* 39, 13-18.
- Kennedy, P.M. & Murphy, M.R., 1988. The nutritional implications of differential passage of particles through the ruminant alimentary tract. *Nutrition Research Reviews* 1, 189-208.
- Klop, A. & De Visser, H., 1994. The effect of different grass silage: maize silage ratios in the ration on feed intake, milk production, rumen fermentation, rumen kinetics and faecal digestibility. ID-DLO report 270. Lelystad, The Netherlands.
- McRae, J.C., Buttery, P.J. & Beaver, D.E., 1988. Nutrient interactions in the dairy cow. In: P.C. Garnsworthy (Ed.), *Nutrition and Lactation in the Dairy Cow*, Butterworths, London, United Kingdom, pp. 55-75.
- Murphy, M.R., Baldwin, R.L. & Koong, L.J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *Journal of Dairy Science* 55, 411-421.
- Neal, H.D.StC., Dijkstra, J. & Gill, M., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model evaluation. *Journal of Nutrition* 122, 2257-2272.
- Owens, F.N. & Goetsch, A.L., 1986. Digesta passage and microbiological protein synthesis. In: L.P. Milligan, W.L. Grovum & A. Dobson (Eds.), *Control of Digestion and Metabolism in Ruminants*, Prentice-Hall, Englewood Cliffs, New Jersey, United States of America, pp. 196-222.
- Poppi, D.P., Gill, M. & France, J., 1994. Integration of theories of intake regulation in growing ruminants. *Journal of Theoretical Biology* 167, 129-145.
- Robinson, P.H., Tamminga, S. & Van Vuuren, A.M., 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.
- Russell, J.B. & Wallaca, R.J., 1988. Energy yielding and consuming reactions. In: P.N. Hobson (Ed.), *The Rumen Microbiological Ecosystem*, Elsevier Science Publishers, London, United Kingdom, pp. 185-215.
- Speckhart, F.H. & Green, W.L., 1976. A Guide to Using CSMP- *the Continuous System Modelling Program*. Prentice-Hall, Englewood Cliffs, New Jersey, United States of America.
- Vaage, A.S., 1992. *Control of particle outflow from the reticulo-rumen*. PhD Thesis, University of Guelph, Guelph, Canada.
- Van Soest, P.J., 1994. *Nutritional Ecology of the Ruminant*. 2nd Edition.. Cornell University Press, New York, United States of America, pp. 476

- Van Vuuren, A.M., Krol-Kramer, F., Van der Lee, R.A. & Corbijn, H., 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. *Journal of Dairy Science* 75, 2215-2225.
- Van Vuuren, A.M., Van der Koelen, C.J. & Vroons-de Bruin, J., 1993. Ryegrass versus corn starch or beet pulp fibre diet effects on digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science* 76, 2692-2700.

Chapter 6

Estimation of the Stoichiometry of Volatile Fatty Acid Production in the Rumen of Lactating Cows

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Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows

Abstract

The purpose of this study was to improve the prediction of the quantity and type of Volatile Fatty Acids (VFA) produced from fermented substrate in the rumen of lactating cows. A model was formulated that describes the conversion of substrate (soluble carbohydrates, starch, hemi-cellulose, cellulose, and protein) into VFA (acetate, propionate, butyrate, and other VFA). Inputs to the model were observed rates of true rumen digestion of substrates, whereas outputs were observed molar proportions of VFA in rumen fluid. A literature survey generated data of 182 diets (96 roughage and 86 concentrate diets). Coefficient values that define the conversion of a specific substrate into VFA were estimated meta-analytically by regression of the model against observed VFA molar proportions using nonlinear regression techniques. Coefficient estimates significantly differed for acetate and propionate production in particular, between different types of substrate and between roughage and concentrate diets. Deviations of fitted from observed VFA molar proportions could be attributed to random error for 100%. In addition to regression against observed data, simulation studies were performed to investigate the potential of the estimation method. Fitted coefficient estimates from simulated data sets appeared accurate, as well as fitted rates of VFA production, although the model accounted for only a small fraction (maximally 45%) of the variation in VFA molar proportions. The simulation results showed that the latter result was merely a consequence of the statistical analysis chosen and should not be interpreted as an indication of inaccuracy of coefficient estimates. Deviations between fitted and observed values corresponded to those obtained in simulations.

Abbreviations: Ac = Acetate, Bc = VFA other than acetate, propionate and butyrate, Bu = Butyrate, Ce = Cellulose, Hc = Hemi-cellulose, Mi = Microbial mass, Pr = Propionate, Pt = Protein, Sc = Soluble and rapidly fermentable non-starch carbohydrates, St = Starch.

Introduction

Volatile fatty acids (VFA) deliver most of the metabolizable energy for a lactating cow. Usually, acetate (Ac), propionate (Pr) and butyrate (Bu) account for more than 95% of the VFA found in rumen fluid. There is an interest to indicate changes in the type of VFA produced with a change in diet because Ac and Bu are primarily used as precursors for long-chain fatty acid synthesis whereas Pr is primarily used as a precursor for glucose. When a

specific diet needs to be evaluated for the type of VFA produced during digestion, the fermentation stoichiometry of distinct substrates must be known. Murphy *et al.* (1982) made an important attempt to derive the coefficients that describe this stoichiometry by a modelling approach. These coefficient values are still considered the best available (Baldwin, 1995) and have been applied in several mechanistic rumen models (Baldwin *et al.*, 1987; Dijkstra *et al.*, 1992). However, the prediction of VFA molar proportions in rumen fluid by these models is inaccurate (Bannink *et al.*, 1997a; Dijkstra & Bannink, 2000; Neal *et al.*, 1992). Subsequent simulation studies demonstrated that this inaccuracy is most likely caused by an inadequate representation of the stoichiometry of VFA production or the rate of VFA absorption (Bannink *et al.*, 1997b).

Murphy *et al.* (1982) used a heterogeneous set of experimental data. Rumen and, if available, duodenal observations were gathered from literature which covered various ruminant species and various levels of feed intake and production. However, hardly any data from lactating cows were used. Therefore, the data used may not be representative of fermentation conditions in the rumen of high-yielding dairy cows and may have generated estimates of stoichiometric coefficients that are inapplicable to the rumen conditions in these animals.

Other studies were performed to improve the accuracy of prediction of VFA molar proportions. Argyle and Baldwin (1988) were the first to consider the effect of rumen pH on the stoichiometry of VFA production. More recently, Pitt *et al.* (1996) derived empirical relationships from *in vitro* fermentation studies of Strobel & Russell (1986), which also included the effect of rumen pH. However, in comparison to the coefficient estimates published by Murphy *et al.* (1982), the prediction of VFA molar proportions seemed not to improve. In another approach by Friggens *et al.* (1998), equations were derived from observations in the sheep rumen that were obtained from experiments specifically designed to quantify the effect of individual chemical components in the diet on VFA molar proportions. Compared to the coefficient values derived by Murphy *et al.* (1982), their equations improved the explanation of observed VFA molar proportions considerably. But, the equations were not evaluated with independent data and, like the coefficient values of Murphy *et al.* (1982), may still give inaccurate prediction of VFA production in lactating cows. The objective of this study was to improve the description of the quantity and the type of VFA produced from fermented substrate in the rumen of lactating cows. A stoichiometric model was described and coefficients for VFA production were estimated by regression of the model against observed rates of rumen digestion in lactating cows only. In addition to the regressions against observed data, simulations were performed to study the effect of random error on the accuracy of coefficient estimates, of fitted rates of VFA production and of fitted VFA molar proportions.

Materials and methods

Description of stoichiometric model

The model used to describe the stoichiometry of VFA production in the rumen was adapted from that published by Koong *et al.* (1975) and applied by Murphy *et al.* (1982). The model describes the condition of steady-state with constant inputs. The notation adopted for model formulation is given in Table 1. Model inputs are the utilization rate of substrate by rumen micro-organisms (U_i , in mol of substrate i/d). Substrate types identified are starch (St), hemi-cellulose (Hc), cellulose (Ce), other carbohydrates (including soluble as well as rapidly fermentable carbohydrates like pectins; Sc) and protein (Pt). Substrate utilized by micro-organisms is incorporated into microbial mass (Mi) or is fermented to generate energy with VFA, and ammonia and several gases, as end products. Although ammonia and fermentation gases are relevant when considering rumen carbon and nitrogen balance, from the perspective of the specific goal of the present study there is no specific interest in representing them. Rate of Mi production (P_{Mi} , in g/d) may be calculated according to Equation [1a] from the rates of substrate utilization, U_i , the fraction of substrate i incorporated into Mi ($f_{i,Mi}$, mol of substrate i incorporated into Mi/mol of substrate i utilized) and a yield factor for the conversion of substrate into Mi ($Y_{Mi,i}$, g of microbial mass/mol of substrate i utilized).

$$P_{Mi} = (f_{Mi,Sc} \times Y_{Mi,Sc} \times U_{Sc} + f_{Mi,St} \times Y_{Mi,St} \times U_{St} + f_{Mi,Hc} \times Y_{Mi,Hc} \times U_{Hc} + f_{Mi,Ce} \times Y_{Mi,Ce} \times U_{Ce} + f_{Mi,Pt} \times Y_{Mi,Pt} \times U_{Pt}) \quad [1a]$$

The rate of VFA production (P_{Vfa} , in mol/d) is then given by Equation [1b], with $Y_{Vfa,i}$ (mol of VFA formed/mol of substrate i utilized) as a yield factor for moles of VFA produced per moles of substrate i converted into VFA

$$P_{Vfa} = [(1 - f_{Mi,Sc}) \times U_{Sc} + (1 - f_{Mi,St}) \times U_{St} + (1 - f_{Mi,Hc}) \times U_{Hc} + (1 - f_{Mi,Ce}) \times U_{Ce} + (1 - f_{Mi,Pt}) \times U_{Pt}] \times Y_{Vfa,i} \quad [1b]$$

When it is assumed that the microbial yield on fermented carbohydrates and protein equals Y_{Mi} , and that $f_{Mi,Sc} = f_{Mi,St} = f_{Mi,Hc} = f_{Mi,Ce} = f_{Mi,Pt} = f_{Mi}$, Equations [1a] & [1b] simplify to [1c] and [1d].

$$P_{Mi} = (U_{Sc} + U_{St} + U_{Hc} + U_{Ce} + U_{Pt}) \times f_{Mi} \times Y_{Mi} \quad [1c]$$

$$P_{Vfa} = (U_{Sc} + U_{St} + U_{Hc} + U_{Ce} + U_{Pt}) \times (1 - f_{Mi}) \times Y_{Vfa,i} \quad [1d]$$

Identifying distinct types of VFA requires a description of the stoichiometry of the conversion of fermented Sc, St, Hc, Ce and Pt into Ac, Pr, Bu, or other VFA, which include valerate and the branched chain VFA (Bc). As a consequence, a distinct stoichiometric coefficient $Y_{Vfa,i}$ is

needed for the fraction of substrate converted into a specific VFA for every substrate-VFA combination. The model contains a total of 20 of these stoichiometric coefficients because it considers five types of substrate and four types of VFA. According to known biochemical pathways of microbial fermentation (Baldwin, 1995), it was assumed that fermentation of 1 mol of monomer equivalents of carbohydrate delivers 2 mol of Ac or Pr, and 1 mol of Bu or Bc, and that 1 mol of monomer equivalent of protein delivers 0.55 mol of carbohydrate monomer equivalents. These stoichiometric constants in combination with the substrate-VFA specific stoichiometric coefficients replace the general yield factor $Y_{Vfa,i}$ used in Equation [1d]. The production rate of individual VFA, as described by Equation [1d], can be replaced then by the following set of equations

$$P_{Ac} = [(Y_{Ac,Sc} \times U_{Sc} + Y_{Ac,St} \times U_{St} + Y_{Ac,Hc} \times U_{Hc} + Y_{Ac,Ce} \times U_{Ce}) \times 2 + Y_{Ac,Pt} \times U_{Pt} \times 1.1] \times (1 - f_{Mi}) \quad [2a]$$

$$P_{Pr} = [(Y_{Pr,Sc} \times U_{Sc} + Y_{Pr,St} \times U_{St} + Y_{Pr,Hc} \times U_{Hc} + Y_{Pr,Ce} \times U_{Ce}) \times 2 + Y_{Pr,Pt} \times U_{Pt} \times 1.1] \times (1 - f_{Mi}) \quad [2b]$$

$$P_{Bu} = [Y_{Bu,Sc} \times U_{Sc} + Y_{Bu,St} \times U_{St} + Y_{Bu,Hc} \times U_{Hc} + Y_{Bu,Ce} \times U_{Ce} + Y_{Bu,Pt} \times U_{Pt} \times 0.55] \times (1 - f_{Mi}) \quad [2c]$$

$$P_{Bc} = [Y_{Bc,Sc} \times U_{Sc} + Y_{Bc,St} \times U_{St} + Y_{Bc,Hc} \times U_{Hc} + Y_{Bc,Ce} \times U_{Ce} + Y_{Bc,Pt} \times U_{Pt} \times 0.55] \times (1 - f_{Mi}) \quad [2d]$$

Although this set of equations is in line with the general interest in prediction of the stoichiometry of VFA production rates, literature on lactating cows only provides data of concentrations and molar proportions of VFA in rumen fluid. Therefore, estimating the stoichiometry of VFA production by regression against literature data requires the model to calculate VFA molar proportions in rumen fluid. For the condition of steady-state with constant inputs, VFA concentrations (C_{Vfa} , mol of VFA/l) can be calculated from P_{Vfa} , the fractional rate of absorption of VFA type j through the rumen wall ($k_{Abs,j}$, in /d), the fractional rate of passage of VFA type j with outflow of rumen fluid ($k_{Out,j}$, in /d), and the liquid volume in the rumen (V_{Ru} , in l) by the following set of equations

$$C_{Ac} = P_{Ac} / ([k_{Abs,Ac} + k_{Out,Ac}] \times V_{Ru}) \quad [3a]$$

$$C_{Pr} = P_{Pr} / ([k_{Abs,Pr} + k_{Out,Pr}] \times V_{Ru}) \quad [3b]$$

$$C_{Bu} = P_{Bu} / ([k_{Abs,Bu} + k_{Out,Bu}] \times V_{Ru}) \quad [3c]$$

$$C_{Bc} = P_{Bc} / ([k_{Abs,Bc} + k_{Out,Bc}] \times V_{Ru}) \quad [3d]$$

The molar proportion of VFA concentrations in rumen fluid ($p_{j,Vfa}$, mol of VFA of type j /mol of total VFA) are defined by

$$p_{Ac,Vfa} = C_{Ac} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) \quad [4a]$$

$$p_{Pr,Vfa} = C_{Pr} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) \quad [4b]$$

$$p_{Bu,Vfa} = C_{Bu} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) \quad [4c]$$

$$p_{Bc,Vfa} = C_{Bc} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) \quad [4d]$$

Table 1. Symbols and notation used in model description

Symbol of entity			
Abs	Absorption from rumen	Out	Fluid outflow
Ac	Acetic acid	Pr	Propionic acid
Bc	Branched chain fatty acids including valerate	Pt	Protein
Bu	Butyric acid	Ru	Rumen
Ce	Cellulose	Sc	Soluble or rapidly fermentable, non-starch, carbohydrates
Hc	Hemi-cellulose	St	Starch
Mi	Microbial mass	Vfa	Volatile fatty acids
Notation	Explanation	Units	
U_i	utilization rate of substrate type i	(mol i /d)	
P_j or P_{Mi}	production rate of VFA type j or Mi	(mol j /d or gMi/d)	
$Y_{j,i}$	yield of VFA type j ¹ or Mi from substrate type i	(mol j /mol i , or g Mi/mol j)	
C_i	concentration of VFA type j	(mol j /l)	
$f_{i,Mi}$	fraction of substrate type i incorporated in Mi	(mol i /mol i)	
$k_{x,j}$	type x fractional flow rate of VFA type j	(/d)	
$p_{j,Vfa}$	molar proportion of VFA type j in total VFA	(mol j / mol total VFA)	

¹ In the case of Ac or Pr to be multiplied by the stoichiometric constant of 2 to obtain the yield of mol Ac or Pr per mol substrate fermented (Equations [2a] & [2b]).

Table 2. Full listing of equations in the model regressed against observed data of rates of substrate fermentation and molar VFA proportions (see Table 1 for explanation of notation and symbols used)

Fitted rate of VFA production was calculated from fitted $Y_{r, \dot{v}a, i}$ ¹ and observed rates of substrate fermented U_j as independent model input according to the following equations.

$$\begin{aligned}
 P_{Ac} &= [(Y_{Ac,Sc} \times U_{Sc} + Y_{Ac,St} \times U_{St} + Y_{Ac,Hc} \times U_{Hc} + Y_{Ac,Ce} \times U_{Ce}) \times 2 + Y_{Ac,Pt} \times U_{Pt} \times 1.1] \\
 P_{Pr} &= [(Y_{Pr,Sc} \times U_{Sc} + Y_{Pr,St} \times U_{St} + Y_{Pr,Hc} \times U_{Hc} + Y_{Pr,Ce} \times U_{Ce}) \times 2 + Y_{Pr,Pt} \times U_{Pt} \times 1.1] \\
 P_{Bu} &= [Y_{Bu,Sc} \times U_{Sc} + Y_{Bu,St} \times U_{St} + Y_{Bu,Hc} \times U_{Hc} + Y_{Bu,Ce} \times U_{Ce} + Y_{Bu,Pt} \times U_{Pt} \times 0.55] \\
 P_{Bc} &= [Y_{Bc,Sc} \times U_{Sc} + Y_{Bc,St} \times U_{St} + Y_{Bc,Hc} \times U_{Hc} + Y_{Bc,Ce} \times U_{Ce} + Y_{Bc,Pt} \times U_{Pt} \times 0.55]
 \end{aligned}$$

Fitted molar proportions of VFA ($p_{Ac, \dot{v}a}, p_{Pr, \dot{v}a}, p_{Bu, \dot{v}a}, p_{Bc, \dot{v}a}$) were regressed against observed values in rumen fluid. Fitted molar proportions of VFA were calculated according to the following equations.

$$\begin{aligned}
 p_{Ac, \dot{v}a} &= P_{Ac} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc}) \\
 p_{Pr, \dot{v}a} &= P_{Pr} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc}) \\
 p_{Bu, \dot{v}a} &= P_{Bu} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc}) \\
 p_{Bc, \dot{v}a} &= P_{Bc} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc})
 \end{aligned}$$

The coefficient values of the three most abundant VFA ($Y_{Ac, i}, Y_{Pr, i}, Y_{Bu, i}$)¹ were fitted with regression whilst $Y_{Bc, i}$ was calculated according to the following equations

$$\begin{aligned}
 Y_{Bc, Sc} &= 1 - Y_{Ac, Sc} - Y_{Pr, Sc} - Y_{Bu, Sc} - Y_{Bc, Sc} \\
 Y_{Bc, St} &= 1 - Y_{Ac, St} - Y_{Pr, St} - Y_{Bu, St} - Y_{Bc, St} \\
 Y_{Bc, Hc} &= 1 - Y_{Ac, Hc} - Y_{Pr, Hc} - Y_{Bu, Hc} - Y_{Bc, Hc} \\
 Y_{Bc, Ce} &= 1 - Y_{Ac, Ce} - Y_{Pr, Ce} - Y_{Bu, Ce} - Y_{Bc, Ce} \\
 Y_{Bc, Pt} &= 1 - Y_{Ac, Pt} - Y_{Pr, Pt} - Y_{Bu, Pt} - Y_{Bc, Pt}
 \end{aligned}$$

¹ In the case of Ac or Pr to be multiplied by the stoichiometric constant of to obtain the yield of mol Ac or Pr per mol substrate fermented (Equations [2a] & [2b]).

Substitution of Equations [3a] through [3d] for C_{Vfa} in both numerator and denominator in Equations [4a] through [4d], results in the following relationships between VFA production rates and molar proportion of VFA concentrations at conditions of steady-state with constant inputs

$$p_{Ac,Vfa} = [P_{Ac} / (k_{Abs,Ac} + k_{Out,Ac})] \times 1 / \phi \quad [5a]$$

$$p_{Pr,Vfa} = [P_{Pr} / (k_{Abs,Pr} + k_{Out,Pr})] \times 1 / \phi \quad [5b]$$

$$p_{Bu,Vfa} = [P_{Bu} / (k_{Abs,Bu} + k_{Out,Bu})] \times 1 / \phi \quad [5c]$$

$$p_{Bc,Vfa} = [P_{Bc} / (k_{Abs,Bc} + k_{Out,Bc})] \times 1 / \phi \quad [5d]$$

with $\phi =$

$$[P_{Ac} / (k_{Abs,Ac} + k_{Out,Ac}) + P_{Pr} / (k_{Abs,Pr} + k_{Out,Pr}) + P_{Bu} / (k_{Abs,Bu} + k_{Out,Bu}) + P_{Bc} / (k_{Abs,Bc} + k_{Out,Bc})].$$

Equations [5a] through [5d] make clear that only for the special case where the sum of $k_{Abs,j}$ and $k_{Out,j}$ is identical for all VFA are molar proportions of VFA production rates identical to those of VFA concentrations found in the rumen, and the following equations hold

$$p_{Ac,Vfa} = C_{Ac} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) = P_{Ac} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc}) \quad [6a]$$

$$p_{Pr,Vfa} = C_{Pr} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) = P_{Pr} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc}) \quad [6b]$$

$$p_{Bu,Vfa} = C_{Bu} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) = P_{Bu} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc}) \quad [6c]$$

$$p_{Bc,Vfa} = C_{Bc} / (C_{Ac} + C_{Pr} + C_{Bu} + C_{Bc}) = P_{Bc} / (P_{Ac} + P_{Pr} + P_{Bu} + P_{Bc}) \quad [6d]$$

Regression model and parameter fitting

The regression model used in the regression studies consisted of Equations [2a] through [2d] and Equations [6a] through [6d]. In Table 2 the full set of equations of the regression model is stated, including the relationships among parameters. The stoichiometric coefficients, $Y_{Vfa,i}$, in Equations [2a] through [2d] were fitted by regression of the equations for $p_{j,Vfa}$ against observed values. Taking account of the influence of $k_{Abs,j}$ and $k_{Out,j}$ on VFA molar proportions requires their values be given as an input to the regression model. However, $k_{Out,j}$ is often not measured and estimates of $k_{Abs,j}$ are even more uncertain. Therefore, like Murphy *et al.* (1982), the pragmatic approach was followed to assume identical $k_{Abs,j}$ and $k_{Out,j}$ for all type of VFA per diet. A distinct set of coefficient estimates was derived for either mainly roughage (R) or mainly concentrate (C) diets. This distinction would cover a systematic difference between R and C diets in fermentation stoichiometry, or in the sum of $k_{Abs,j}$ and $k_{Out,j}$. Nevertheless, the effects of a systematic difference between R and C

diets in the degree at which the sum of $k_{Abs,j}$ and $k_{Out,j}$ differs for individual VFA (Dijkstra *et al.*, 1993; Sutton, 1985) will still be included in the $Y_{Vfa,i}$ estimates obtained.

Further, the assumption was made that partitioning of utilized substrate over microbial growth and VFA production was identical for all substrate types. This assumption resulted in a common factor $(1-f_{Mi})$ in Equations [2a] through [2d] and allowed for elimination of this factor from the model, and thereby of its effect on fitted $Y_{Vfa,i}$ values. As a consequence, observed rates of true substrate digestion could be used as model input without considering microbial growth.

Nonlinear regression techniques were used to estimate the stoichiometric coefficients ($Y_{Vfa,i}$ in Equations [2a] through [2d]) by minimizing the difference between observed and predicted VFA molar proportions in rumen fluid ($p_{j,Vfa}$ in Equations [6a] through [6d]), with observed rates of true substrate digestion as independent inputs. Regressions were performed with the procedure FITNONLINEAR of GENSTAT (1993). This procedure applies the NewtonRaphson as the default algorithm to optimize the model specified and the convergence criterion was 0.0001. Both the type of algorithm (GaussNewton, NewtonRaphson or FletcherPowell) and the convergence criterion applied had negligible effects on the regression result. Lower and upper values of $p_{j,Vfa}$ were selected of 0.0 and 1.0, and the initial values of $p_{j,Vfa}$ were 0.5, 0.2 and 0.2 for the yield of Ac, Pr and Bu from fermented substrates, respectively. Also the choice of initial values had no effect on the regression results however. The maximum number of iterations allowed was 30, but on average the convergence criterion of 0.0001 was met already after 5 iterations.

All $Y_{Vfa,i}$ values were restricted to a value between 0 and 1. The value of $(Y_{Ac,i} + Y_{Pr,i} + Y_{Bu,i} + Y_{Bc,i})$ was set to unity because the proportions of production rates of individual VFA from a specific type of substrate i sum up to the total rate of VFA production from that substrate. The coefficients values of the three most abundant VFA ($Y_{Ac,i}$, $Y_{Pr,i}$ and $Y_{Bu,i}$) were fitted and $Y_{Bc,i}$ was calculated by difference from unity $(1 - Y_{Ac,i} - Y_{Pr,i} - Y_{Bu,i})$.

Observed data. Data were derived for 182 treatments in 47 rumen digestion trials with Friesian-breeds of high-yielding cows, mostly Holstein–Friesian, in several stages of lactation (Aldrich *et al.*, 1993; Beever *et al.*, 1988; Benchaar *et al.*, 1991; Calsamiglia *et al.*, 1995; Cameron *et al.*, 1991; Christensen *et al.*, 1993; Cunningham *et al.*, 1993, 1994, 1996; De Visser *et al.*, 1991, 1993, 1997; Erasmus *et al.*, 1994; Feng *et al.*, 1993; Ferlay *et al.*, 1992; Haïmoud *et al.*, 1995; Herrera-Saldana *et al.*, 1990; Khorasani *et al.*, 1996; Klop & De Visser, 1994; Klusmeyer *et al.*, 1990, 1991a, 1991b; Kung *et al.*, 1983; Lynch *et al.*, 1991; Mansfield & Stern, 1994; McCarthy *et al.*, 1989; McNiven *et al.*, 1995; Overton *et al.*, 1995; Palmquist *et al.*, 1993; Pantoja *et al.*, 1995; Pena *et al.*, 1986; Poore *et al.*, 1993; Robinson *et al.*, 1987, 1991; Robinson & Kennelly, 1990; Rohr *et al.*, 1986; Santos *et al.*, 1984; Stern *et al.*, 1983; Stokes *et al.*, 1991; Sutton *et al.*, 1980; Tamminga *et al.*, 1983; Tice *et al.*, 1993; Van Vuuren *et al.*, 1993a, b; Waltz *et al.*, 1989; Windschitl & Stern, 1988; Zerbini *et al.*, 1988).

Treatments that involved the addition of compounds with a potential effect on microorganisms (e.g. antibiotics or more specific inhibitors) were omitted from the data set. Reported molar proportions of VFA in rumen fluid ($p_{j,vfa}$) and rates of truly digested substrate (g of substrate/d) were used. From the latter, carbohydrate or protein monomer equivalents were calculated (U_i , mol of carbohydrate or protein/d) by assuming a molecular weight of 162 g and 110 g per mol of carbohydrate and protein monomer equivalents. Missing values for feed composition were calculated from estimates in feeding tables and reported feed composition in digestion trials with similar feeds. The intake rate of protein (g of protein/d) was estimated by subtracting the non-protein nitrogen component (ammonia ingested with urea treated diets or with silages) from reported total intake of nitrogen. If not reported, truly digested protein (mol of protein/d) was estimated as the difference between the intake rate of protein and the duodenal outflow of feed protein (outflow of feed protein assumed equal to outflow of total non-ammonia crude protein minus microbial crude protein). Similarly, truly digested organic matter (g of organic matter/d) was estimated by intake rate of organic matter minus duodenal outflow of feed organic matter (outflow of feed organic matter = outflow of organic matter – outflow of microbial organic matter). The dietary fraction not accounted for by component analysis (dry matter – ash – crude fat – starch – neutral detergent fibre – non-ammonia crude protein) was assumed to be soluble or rapidly fermentable (non-starch) carbohydrate. The latter fraction was assumed to be digested completely in the rumen. This fraction also included fermentation end products in silages because lactate as the main constituent (70–80%) also serves as substrate for microbial fermentation in the rumen. In case the diet consisted of almost exclusively silage material (De Visser *et al.*, 1991, 1993), the analysed fraction of VFA was subtracted. Alternative to these calculations, the rate of digestion of the fraction not accounted for in organic matter analysis was also estimated by subtracting reported values of true digestion rates of the individual components in organic matter from the reported rate for organic matter. In this way, both data component analysis in combination with observed feed intake, and data on rumen digestion were consulted. In case of inconsistencies, estimates were based on (1) measurements obtained in the period most closely matching the period during which VFA samples were collected, or (2) reported values of feed intake and component analysis because these were considered less susceptible to measurement error than duodenal flow measurement.

Method testing and simulations. Before $Y_{Vfa,i}$ estimates were derived from *in vivo* data, simulation studies were performed to test the accuracy of the statistical method used. The stoichiometric model described above, with observed rates of utilization of substrate U_i as model inputs, was used to create a simulated data set of $p_{j,vfa}$ values from an arbitrarily chosen set of values for the $Y_{Vfa,i}$ coefficients. Subsequently, on the same U_i as independent model inputs, the model was regressed against the simulated $p_{j,vfa}$. The correspondence between fitted $Y_{Vfa,i}$ values and the values that were originally used to create the simulated

data set indicated the accuracy of the estimation method. The effect of random error on the accuracy of fitted $Y_{Vfa,i}$ values was simulated by the addition of random error with a normal distribution to P_{Vfa} (mean value of 0.0 and standard deviation of either 5.0 or 10.0 mol VFA/d) during generation of the simulated data sets of $p_{j,Vfa}$ values. This procedure was repeated in ten runs and from the fitted $Y_{Vfa,i}$ their means and standard errors were calculated. A different seed for random number generation (GENSTAT, 1993) with every run allowed the generation of ten independent data sets.

Fitting stoichiometric coefficients from in vivo data. After testing the accuracy of the statistical method with simulations, *in vivo* estimates of $Y_{Vfa,i}$ coefficients were fitted from observed values of $p_{j,Vfa}$ and U_i . A separate set of $Y_{Vfa,i}$ was fitted for C diets (up to 50% roughage, n = 86) and R diets (more than 50% roughage, n = 96) following the approach of Murphy et al. (1982). Differences among the experimental trials may have caused a study effect which interferes with parameter estimation from observed data (St-Pierre, 2001). However, in comparison to a total number of 15 model parameters that need to be estimated, each experiment delivered a very small number of data (on average just four combinations of data on rumen digestion and VFA molar proportions). Further, the regression model in the present study does not contain intercept parameters, and hence no interference of different intercept values among studies with the estimation of the $Y_{Vfa,i}$ coefficient values exists. For these reasons, in the present regression analysis no attempts were made to represent study effects. This means that if a significant study effect does exist, the estimation of parameters of regression models can be biased and the SE values of $Y_{Vfa,i}$ estimates will be inflated.

Also weighting of data was considered to account for differences among studies in SEM values reported for the rumen VFA observations. However, the variation in reported SEM values among studies was considered small in that values mostly did not differ more than by a factor of two. Moreover, reported SEM values of individual types of VFA (variation also differs by a factor of two) within a study differed to the same extent as the differences among studies. Also the order of the SEM size for individual VFA differed strongly among studies. For these reasons, the relevance of using reported SEM values for weighting data of VFA was considered low, and no weighting was applied.

Comparison of fitted and observed values. The regression error was assessed by calculation of the mean squared difference between fitted and observed values of $p_{j,Vfa}$ (MSPE). The size of the error was calculated as the root MSPE and expressed as a percentage of the mean observed values. The MSPE was decomposed into three components; overall bias, deviation from the regression slope from one, the disturbance proportion indicating random deviation which can not be accounted for by bias or regression deviation (Bibby & Toutenburg, 1977).

Statistical tests. Statistical tests were performed to test for the significance of differences in $Y_{Vfa,i}$ estimates. The difference in $Y_{Vfa,i}$ estimates for two distinct types of substrate was

investigated by using the *F-test* to test the significance of the increase in the sum of squared differences between fitted and observed $p_{j,Vfa}$ when the fitted value of $Y_{Vfa,i}$ for these two types of substrate was kept identical (for R diets $df1 = 1$ and $df2 = 329$, and for C diets $df1 = 1$ and $df2 = 369$ for numerator and denominator of the *F-test*). The difference in the estimated value of a single $Y_{Vfa,i}$ coefficient between R and C diets was investigated by regressing the model simultaneously, but independently (separate sets of $Y_{Vfa,i}$ estimates for the data set of R diets and that of C diets), against data in a single regression run. The *F-test* ($df1 = 1$ and $df2 = 698$) was used to test for the significance of the extra sum of squared differences between fitted and observed $p_{j,Vfa}$ when the estimate of a single $Y_{Vfa,i}$ coefficient was kept identical for the R and C diets.

Table 3. Summary of experimental data ¹

	R diets				C diets			
	Mean	Median	SD	Range	Mean	Median	SD	Range
DMI (kg/d)	18.0	17.5	2.9	13.0–4.9	19.0	19.5	3.9	5.3–26.7
<i>Diet composition (g/kg of DMI)</i>								
Soluble and rapidly fermentable carbohydrates ²	108	90	85	1–319	98	84	64	1–421
Starch	250	291	146	4–481	270	288	110	6–539
NDF	359	353	65	195–484	339	340	63	148–483
N	26.1	25.5	4.4	18.2–40.4	28.5	28.0	3.6	21.0–47.4
Roughage percentage (%)	63.7	59.7	12.8	51.4–94.0	41.2	45.0	10.4	0.0–50.0
Cellulose in NDF (%)	55.0	53.6	6.9	41.0–73.0	56.7	56.2	8.4	26.4–96.3
<i>Model inputs (independent variables)</i>								
<i>Truly digested in rumen (kg/d)</i>								
Soluble and rapidly fermentable carbohydrates ²	1.83	1.60	1.31	0.02–4.86	1.78	1.67	1.13	0.02–6.71
Starch	2.64	2.88	1.60	0.00–5.80	3.11	3.16	1.47	0.00–6.71
Hemi-cellulose	1.56	1.44	0.71	0.47–3.11	1.46	1.33	0.71	0.00–4.44
Cellulose	1.51	1.49	0.62	0.19–3.21	1.59	1.51	0.70	0.21–4.05
Protein ³	1.65	1.69	0.50	0.79–2.90	1.80	1.77	0.57	0.06–3.34
<i>Observed molar VFA proportions in rumen fluid (mmol/mmol total VFA)</i>								
Acetate	0.624	0.631	0.037	0.552–0.702	0.609	0.613	0.040	0.434–0.701
Propionate	0.214	0.210	0.029	0.153–0.299	0.231	0.223	0.042	0.157–0.410
Butyrate	0.123	0.123	0.013	0.099–0.162	0.120	0.117	0.018	0.081–0.169
Other VFA	0.039	0.038	0.009	0.024–0.058	0.039	0.041	0.013	0.000–0.078

¹ Total number of 182 treatments for which observed values were available.

² Soluble or rapidly fermentable (non-starch) carbohydrates, estimated as organic matter minus starch, hemi-cellulose, cellulose, protein, fat and ash.

³ Non-ammonia N ingested with feed N plus duodenal outflow of microbial N minus duodenal outflow of non-ammonia. Protein values calculated by multiplying N by 6.25.

Table 4. Correlation between rumen observations of amount of substrate truly digested (U_i , mol of monomer equivalents/d) and VFA molar proportions ($p_{j,vfa}$, mol of VFA/mol total VFA)¹

	Sc	St	Hc	Ce	Pt	Ac	Pr	Bu	Bc
Sc	1.000								
St	-0.622*	1.000							
Hc	0.150*	-0.109	1.000						
Ce	0.394*	-0.257*	0.356*	1.000					
Pt	0.286*	0.064	0.351*	0.364*	1.000				
Ac	0.345*	-0.504*	-0.027	0.132*	-0.089	1.000			
Pr	-0.417*	0.602*	-0.057	-0.178*	0.114	-0.819*	1.000		
Bu	0.267*	-0.283*	0.269*	0.184*	0.033	-0.145*	-0.384*	1.000	
Bc	-0.149*	0.089	-0.103	-0.111	-0.035	-0.412*	-0.032	0.325*	1.000

* Statistical significance of linear correlation, $p < 0.05$, $n = 182$.

¹ See Table 1 for explanation of abbreviations.

Results

Data were gathered from the literature for a wide range of experimental conditions and feed ingredients, which is illustrated by the range of feed intake, diet composition, roughage content of the diet, and amounts of substrate truly digested in the rumen (Table 3). Despite the variety of experimental conditions covered by the reports selected from literature, observed rates of digestion of soluble carbohydrate and starch were negatively correlated, as well as the observed molar proportions of Ac and Pr concentrations in rumen fluid (Table 4). However, these correlations are probably inevitable and the current data set was considered the best that can be achieved to derive general estimates of the $Y_{Vfa,i}$ coefficients in lactating cows.

Method testing and simulation results

With no random error added during the generation of simulated data sets of $p_{j,vfa}$, the values of the $Y_{Vfa,i}$ coefficients fitted from these $p_{j,vfa}$ were identical to the coefficient values that were originally used to simulate the data (Table 5), even for the small coefficient values of Bc production. The standard error of the fitted values was none. Comparison of fitted against simulated values of $p_{j,vfa}$ and P_{Vfa} demonstrated a perfect fit with the model explaining 100% of variation. Addition of normal distributed random error (either 0.0 ± 5.0 or 0.0 ± 10.0 mol of VFA/d) during simulation, introduced substantial error in the fitted $Y_{Vfa,i}$ values. The percentage of deviation of fitted from prescribed values of $Y_{Vfa,i}$ was, on average, smallest for the largest values of Ac production (up to 1%; Table 5) and largest for the smallest coefficient values of Bu and Bc production (up to 17%; Table 5). Also as a consequence of error in fitted Y_{Vfa} error was introduced in fitted $p_{j,vfa}$ i . Doubling the size of random error added during

simulation (from 0.0 ± 5.0 to 0.0 ± 10.0 mol of VFA/d) increased the error of fitted $Y_{Vfa,i}$ values and increased their standard error (Table 5). The increase in the size of the deviations was relatively small, however, compared to the error introduced by adding a random error of 0.0 ± 5.0 mol of VFA/d during simulation. Also the sign of deviations between fitted and prescribed values of $Y_{Vfa,i}$ remained the same. This can be explained by the identical random numbers (identical seed numbers used) that were generated with the two sizes of random error introduced during subsequent simulation runs. The size of the random error introduced (0.0 ± 10.0 mol of VFA/d) was large in comparison to the size of error expected for observed data (Figures 1 & 2). Nevertheless, estimated $Y_{Vfa,i}$ remained not significantly different from the original values used during simulation (Table 5). The model explained only a small fraction of the simulated variation in $p_{j,Vfa}$ and was unable to fit the smallest and largest values in the full range of simulated $p_{j,Vfa}$ values of individual VFA (Figures 1A & 2A). In contrast, fitted P_{Vfa} did cover the full range of the simulated data and the error introduced was randomly distributed around the simulated values (Figures 1B & 2B).

Estimates of stoichiometric coefficients in vivo

A single set of $Y_{Vfa,i}$ coefficients fitted for all diets, as well as a separate set for R diets (Figure 3A) and for C diets (Figure 3B), resulted in deviations between fitted and observed $p_{j,Vfa}$ that resembled those obtained in the simulation studies. Even the fitting behaviour for individual diets established during simulations seemed to be reproduced when the fitting the *in vivo* data (Figures 1 & 2 vs. Figure 3). Deviations between fitted and observed $p_{j,Vfa}$ were smallest for Pr, on C diets in particular. The percentage of observed variation of $p_{Ac,Vfa}$, $p_{Pr,Vfa}$, $p_{Bu,Vfa}$ and $p_{Bc,Vfa}$ that could be accounted for by the model was 35%, 38%, 17% and 12%, respectively, for R diets and 25%, 45%, 30% and 6%, respectively, for C diets. Despite these small percentages of variation explained by the model, the deviation of fitted from observed $p_{j,Vfa}$ could be accounted to random variation for 100%. Linear regression of fitted against observed values did not significantly differ from the line of identity (legend Figure 3), even for $p_{Bc,Vfa}$, which indicates that there was no systematic error.

Whether a single set of $Y_{Vfa,i}$ coefficients for all diets, or separate sets for R diets and C diets were estimated, the model always explained approximately the same percentage of observed variation of $p_{j,Vfa}$ (about 99% of the variation, Figure 3A & 3B). Nevertheless, a distinction between R and C diets significantly reduced the residual sum of squares and a number of $Y_{Vfa,i}$ estimates were found to be significantly different between R and C diets (Table 6). For C diets, the conversion of Sc to Ac and Pr was significantly ($p < 0.05$) lower and higher, respectively, compared to that on R diets; that of St to Pr and Bu was significantly ($p < 0.05$) higher and lower, respectively; that of Ce to Ac and Pr tended ($p < 0.1$) to be higher and lower, respectively. Although of a similar magnitude the coefficient estimates for the conversions of Pt into Ac and Pr and of Hc into Pr were lower, the standard errors were large and differences remained insignificant.

Table 5. Prescribed and fitted values of stoichiometric coefficients¹ for VFA production from fermented substrate in simulation studies^{2,3,4}

Substrate type	VFA type	Ac	Pr	Bu	Bc
Sc	Original ⁵	0.5954	0.1130	0.2452	0.0464
	Fitted, no error added ⁶	0.5954	0.1130	0.2452	0.0464
	Fitted, N(0,5) ⁷	0.5906 (0.0184)	0.1154 (0.0145)	0.2411 (0.0238)	0.0529 (0.0249)
	Fitted, N(0,10) ⁷	0.5878 (0.0256)	0.1164 (0.0201)	0.2381 (0.0331)	0.0577 (0.0346)
St	Original ⁵	0.4856	0.2579	0.1858	0.0707
	Fitted, no error added ⁶	0.4856	0.2579	0.1858	0.0707
	Fitted, N(0,5) ⁷	0.4856 (0.0115)	0.2671 (0.0092)	0.1739 (0.0150)	0.0734 (0.0157)
	Fitted, N(0,10) ⁷	0.4850 (0.0116)	0.2707 (0.0128)	0.1679 (0.0209)	0.0763 (0.0218)
Hc	Original ⁵	0.4869	0.1519	0.3100	0.0513
	Fitted, no error added ⁶	0.4868	0.1519	0.3100	0.0513
	Fitted, N(0,5) ⁷	0.4940 (0.0276)	0.1415 (0.0220)	0.3044 (0.0359)	0.0602 (0.0375)
	Fitted, N(0,10) ⁷	0.4951 (0.0384)	0.1368 (0.0305)	0.3018 (0.0500)	0.0663 (0.0522)
Ce	Original ⁵	0.5955	0.1491	0.2037	0.0517
	Fitted, no error added ⁶	0.5955	0.1491	0.2037	0.0517
	Fitted, N(0,5) ⁷	0.5886 (0.0312)	0.1461 (0.0247)	0.2178 (0.0405)	0.0476 (0.0423)
	Fitted, N(0,10) ⁷	0.5840 (0.0435)	0.1433 (0.0344)	0.2236 (0.0564)	0.0491 (0.0589)
Pt	Original ⁵	0.5104	0.2620	0.1062	0.1214
	Fitted, no error added ⁶	0.5104	0.2620	0.1062	0.1214
	Fitted, N(0,5) ⁷	0.5135 (0.0453)	0.2482 (0.0362)	0.1218 (0.0591)	0.1164 (0.0616)
	Fitted, N(0,10) ⁷	0.5174 (0.0631)	0.2422 (0.0504)	0.1281 (0.0823)	0.1186 (0.1173)

¹ In the case of Ac or Pr to be multiplied by the stoichiometric constant of 2 to obtain the yield of mol Ac or Pr per mol substrate fermented (Equations [2a] & [2b]).

² See Table 1 for explanation of abbreviations.

³ Mean coefficient value (standard error within parentheses) fitted from 10 simulated data sets generated in 10 independent runs. With every run a different seed was used for random number generation (Section 2).

⁴ Pair-wise differences between original and fitted coefficient values were never significant.

⁵ Coefficient values in creating the simulated data set. Values were arbitrarily set equal

to values obtained from fitting the simulated data set. Values were arbitrarily set equal

⁶ Standard error of coefficient estimates too small to be estimated.

⁷ Addition of normal distributed random error to simulated values with mean value of 0.0 mol of VFA/d and standard deviation of 5.0 or 10.0 mol of VFA/d indicated by N(0,5) and N(0,10) respectively (Section 2).

In addition to testing the difference between coefficient estimates for R and C diets, the importance of distinguishing between the five types of substrate was tested. These tests indicated a significant difference in $Y_{Ac,i}$, $Y_{Pr,i}$ and $Y_{Bu,i}$ estimates between substrate types, for R diets as well as for C diets (Table 6). Therefore, a distinction between Sc, St, Hc, Ce and Pt was relevant to explain the observed variation in $p_{Ac,Vfa}$, $p_{Pr,Vfa}$ and $p_{Bu,Vfa}$. For $p_{Bc,Vfa}$ with C diets only, there was an indication of a higher $Y_{Bc,Pt}$ estimate (Table 6).

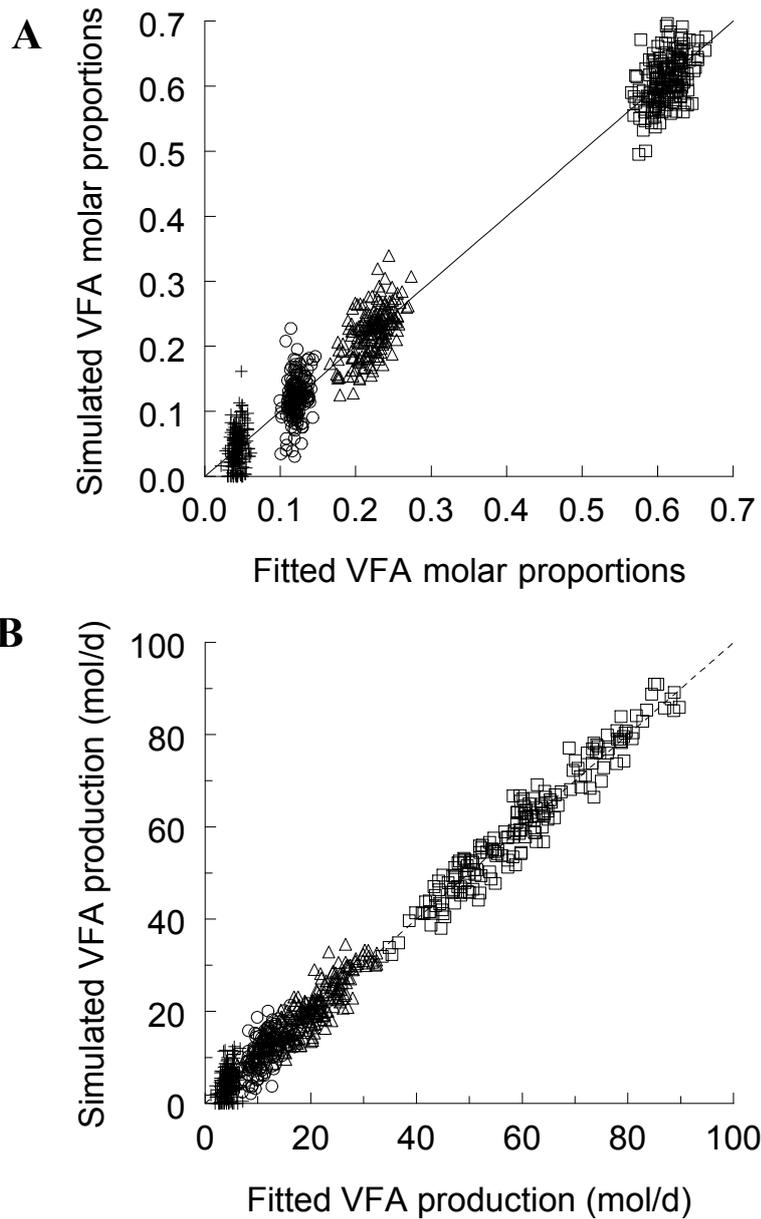


Figure 1. Regression of the stoichiometric model against a simulated data set of molar VFA proportions ($p_{j,Vfa}$) when normal distributed random error of 0.0 ± 10.0 mol/d was added to simulated rates of VFA production (P_{Vfa}). Fitting results for (A) $p_{j,Vfa}$, (B) P_{Vfa} . The graph shows the results of a typical simulation run (see Section 2 for explanation of simulation runs; \square , acetate; \triangle , propionate; \circ , butyrate; $+$, branched chain fatty acids, including valerate). Overall bias in prediction, deviation from a regression slope of one, and disturbance proportion (see Section 2) attributed to MSPE with 0.0%, 0.0% and 100.0%, respectively, for every type of VFA.

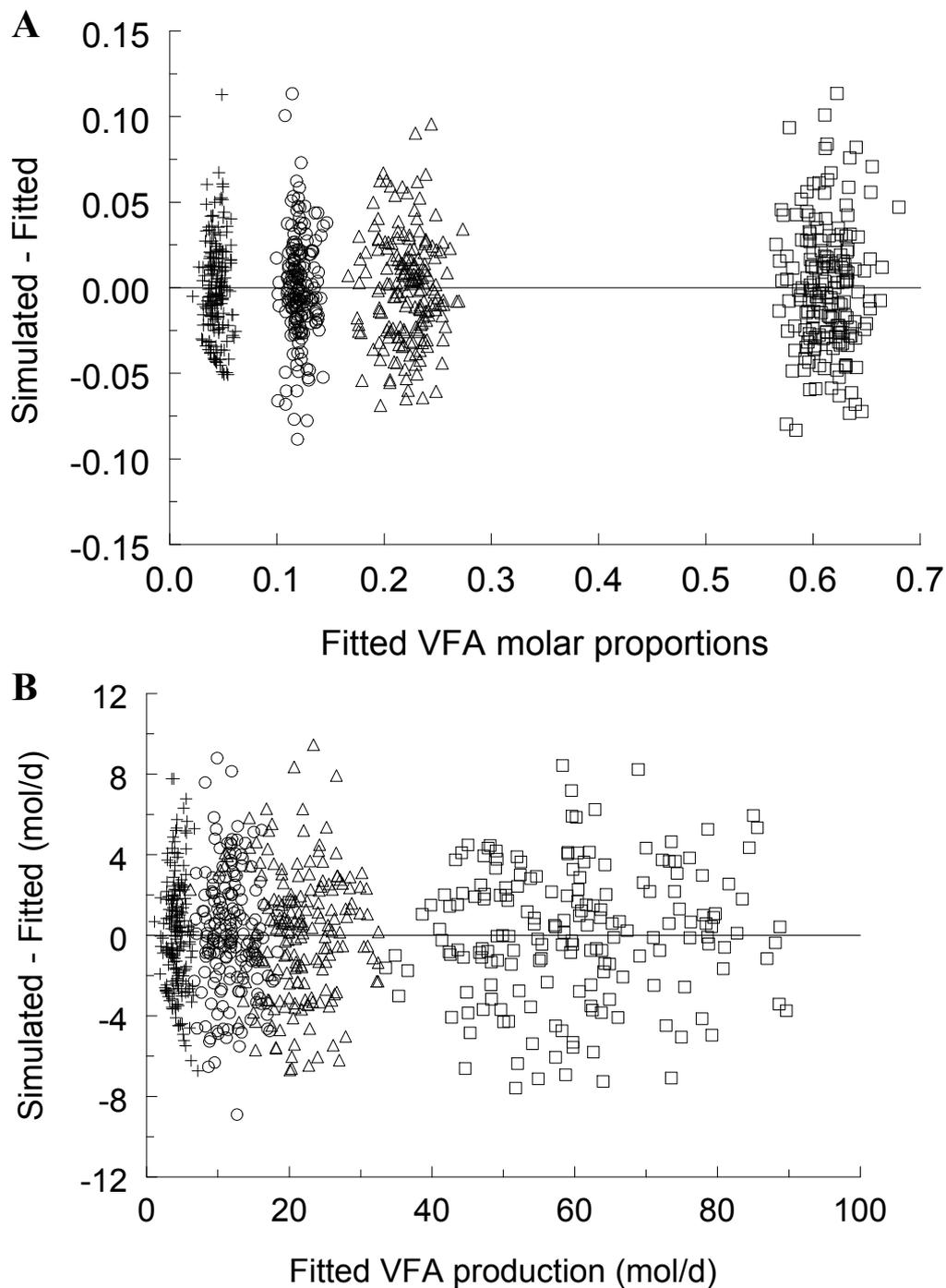


Figure 2. Regression of the stoichiometric model against a simulated data set of VFA molar proportions ($p_{j,Vfa}$) when normal distributed random error of 0.0 ± 10.0 mol/d was added to simulated rates of VFA production (PVfa). Deviations between simulated and fitted values of (A) $p_{j,Vfa}$, (B) P_{Vfa} . The graph shows the results of a typical simulation run (see Section 2 for explanation of simulation runs; \square , acetate; \triangle , propionate; \circ , butyrate; $+$, branched chain fatty acids, including valerate). Overall bias in prediction, deviation from a regression slope of one, and disturbance proportion (see Section 2) attributed to MSPE with 0.0%, 0.0% and 100.0%, respectively, for every type of VFA, both for the data set of roughage diets and that of concentrate diets.

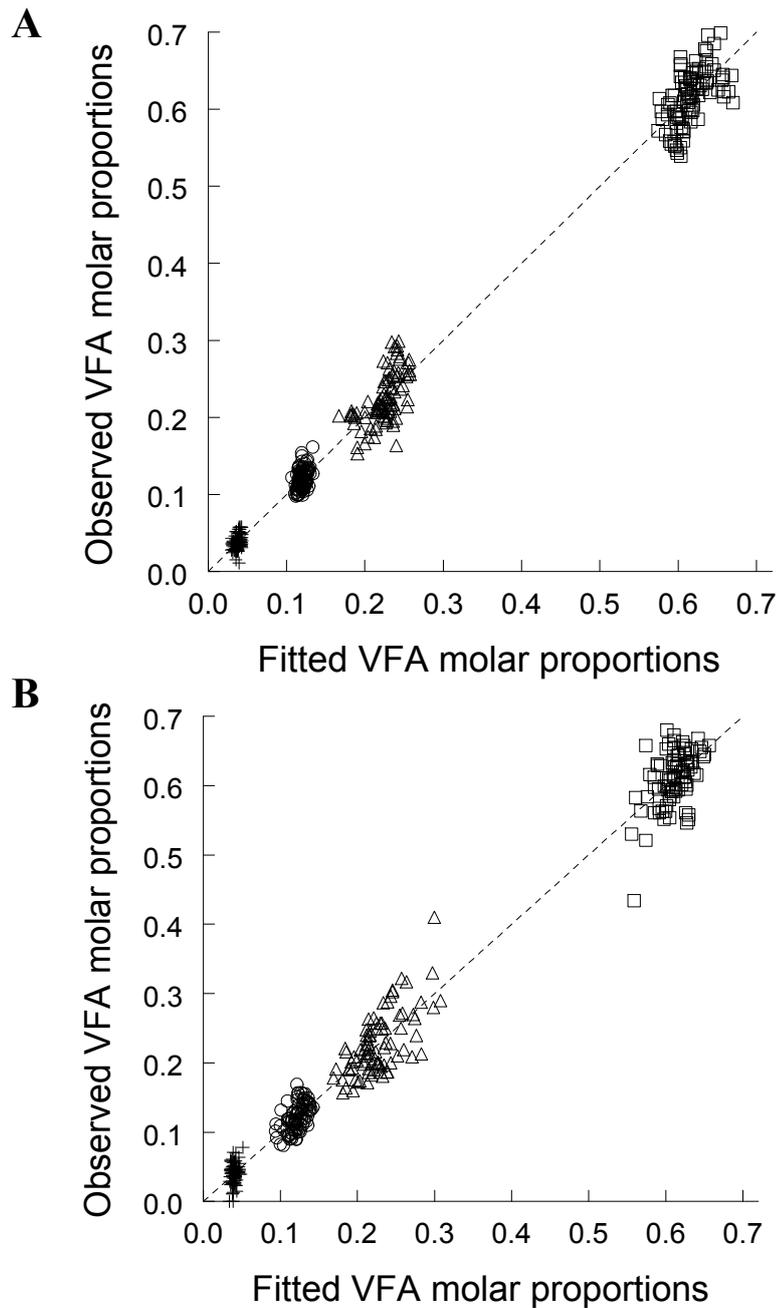


Figure 3. Results of fitting to observed VFA molar proportions in rumen fluid ($p_{j,Vfa}$; \square , acetate; \triangle , propionate; \circ , butyrate; $+$, branched chain fatty acids, including valerate). (A) Separate fit for predominantly roughage diets, (B) separate fit for predominantly concentrate diets.

The mean of observed values mean and the root of the mean square prediction error, expressed as a percentage of the observed mean, of acetate, propionate, butyrate and branched chain fatty acids was 0.617 and 4.80%, 0.225 and 11.41%, 0.120 and 9.64%, and 0.038 and 22.47%, respectively, for roughage diets (A), and 0.611 and 5.84%, 0.227 and 14.60%, 0.122 and 13.87%, and 0.040 and 34.71% respectively for concentrate diets (B).

Overall bias in prediction, deviation from a regression slope of one, and disturbance proportion (see Section 2) attributed to MSPE with 0.0%, 0.0% and 100.0%, respectively, for every type of VFA, both for the data set of roughage diets and that of concentrate diets.

Discussion

Chosen representation

The stoichiometric model used in the present study only considers the effects of type of substrate and rate of fermentation on VFA molar proportions. This is a much simplified representation of rumen fermentation and neglects several aspects that are important determinants of rumen fermentation stoichiometry. Not explicitly described in the present model are (i) the variation in the presence of distinct types of fermenting micro-organisms in the rumen and their distinct fermentation stoichiometry as a result of their enzymatic characteristics (types of substrate utilized and types of VFA produced as endproducts; Russell, 1984), (ii) the effect of the variation in the partitioning of utilized substrate into microbial synthesis and production of VFA and variation in the rate of microbial synthesis (Counotte, 1981; Hespell & Bryant, 1979, Russell, 1984), (iii) the effect of variation in VFA absorption rates (Dijkstra *et al.*, 1993), and (iv) the effect of variation in specific factors or growth conditions that influence microbial metabolism and the type of VFA produced. Although these additional aspects are important to understand rumen fermentation stoichiometry, and could be included in the present model, their representation would result in a complicated mechanistic model that requires inputs that are probably not available from the majority of rumen digestion trials. Murphy *et al.* (1982) applied a rumen model that suited the purpose of estimating the effect of substrate type on $p_{j,Vfa}$ from available observations of rumen fermentation. In the present study, this model was simplified in order to remove model elements that proved least helpful in explaining $p_{j,Vfa}$ data and to improve the fitting behaviour of the model (e.g. preventing negative $Y_{Vfa,i}$ estimates). The model described by Koong *et al.* (1975) (Baldwin, 1995) and applied by Murphy *et al.* (1982) considers a separate constant fraction of Ac and Bc produced from fermented Pt, apart from Ac and Bc originating from pyruvate produced. In the present study, these constant fractions were omitted and an average production of 1.1 mol of pyruvate units from 1 mol of Pt fermented was assumed instead (Equations [2a] through [2d]). Further, the model applied by Murphy *et al.* (1982) included the efficiency of microbial growth as an additional stoichiometric coefficient. In their approach, a single value was fitted for all dietary treatments. It can be demonstrated from the equations given by Koong *et al.* (1975) that the partitioning of fermented substrate into microbial matter and VFA were effectively almost the same for all types of carbohydrates. These observations led us to simplify the model by assumption of an identical partitioning for all substrate types and omission of the efficiency of microbial growth as a parameter from the model. Otherwise, the present approach still compares to that of Murphy *et al.* (1982) and therefore allowed for a similar type of analysis of observed $p_{j,Vfa}$.

Implications of simulation results

The simulation results demonstrate that the statistical procedure is in principle well capable of deriving correct estimates of the 20 stoichiometric coefficients of VFA production contained in the model. Introduction of normal distributed random error seemed to cause a systematic over- and under-prediction of small and large values of VFA proportions, respectively, with an accurate prediction of the mean but only a small fraction of the simulated variation in VFA proportions explained by the model (Figures 1 & 2). Despite these results for VFA molar proportions, coefficient estimates did not significantly differ from the correct values and also a much higher fraction of the simulated variation in VFA production rates was explained than of VFA molar proportions. It is concluded that coefficient estimates remain relevant even though with the present statistical approach only a small fraction of the variation in VFA molar proportions is explained. The regression results obtained for the *in vivo* data showed that experimental error had a similar effect. Both with *in vivo* data and with simulated data, no systematic errors were established and analysis of deviation between observed and fitted values (Figures 1, 2 & 3) indicated nonexplained variation was attributable for 100% to random error. Comparing results for *in vivo* data with those for simulated data indicates that a standard deviation of 10 mol/d is most realistic to account for fluctuations in the rate of Ac and Pr production during the day, whereas for Bu and Bc production 5 mol/d is more appropriate. Similar deviations between fitted and observed VFA proportions in previous work on the stoichiometry of VFA production support the results of the present study (Baldwin, 1995; Murphy *et al.*, 1982; Pitt *et al.*, 1996). However, the cause of these deviations that was reestablished in the present study seems not to be recognized in literature. The major cause was the calculation of VFA proportions as a ratio by dividing concentration of a specific VFA by total VFA concentration (Equations [4a] through [4d]) and the sum of all VFA proportions being unity. By this, calculated VFA proportions become more interdependent than calculated VFA production rates (Equations [2a] through [2d]). For this reason, deviations of VFA production rates are much smaller than those of VFA molar proportions (Figures 1 & 2). In another simulation study (results not shown), with the calculation of VFA molar proportions omitted from the model, fitting results for VFA production rates were similar to those shown here. As clearly illustrated by the simulation results, observations of VFA production rates are to be preferred in analyzing stoichiometry of the production of specific type of VFA from of a type of substrate fermented. However, reports in the literature on the direct measurement of VFA production rates in the rumen of lactating cows are almost lacking. Consequently, the rate of rumen substrate degradation remains by necessity the independent driving variable from which VFA production rates must be calculated. Such calculations require appropriate estimates of VFA molar proportions produced from fermented substrate.

Table 6. Estimates of stoichiometric coefficients for VFA production from fermented substrate ($Y_{Vfa,i}$)¹ from observations of roughage diets (R) and concentrate diets (C)²

Substrate type	Diet type ⁴	VFA type ³			
		Ac	Pr	Bu	Bc
Sc	R	0.6440 (0.0218) ^{a**}	0.0801 (0.0170) ^{a**}	0.2389 (0.0281) ^{ab}	0.0370 (0.0294) ^a
St	R	0.4899 (0.0138) ^b	0.2159 (0.0109) ^{b**}	0.2135 (0.0178) ^{ab**}	0.0807 (0.0186) ^a
Hc	R	0.4417 (0.0377) ^b	0.1773 (0.0299) ^b	0.3174 (0.0489) ^a	0.0635 (0.0511) ^a
Ce	R	0.5583 (0.0390) ^{ab*}	0.2027 (0.0311) ^{b*}	0.1678 (0.0507) ^{ab}	0.0712 (0.0530) ^a
Pt	R	0.5636 (0.0550) ^{ab*}	0.2930 (0.0436) ^b	0.0816 (0.0714) ^b	0.0617 (0.0746) ^a
Sc	C	0.5293 (0.0335) ^c	0.1552 (0.0265) ^c	0.2553 (0.0435) ^c	0.0603 (0.0455) ^c
St	C	0.4874 (0.0207) ^c	0.3118 (0.0168) ^d	0.1484 (0.0272) ^d	0.0524 (0.0283) ^c
Hc	C	0.5115 (0.0428) ^c	0.1178 (0.0342) ^c	0.3191 (0.0557) ^c	0.0516 (0.0584) ^c
Ce	C	0.6829 (0.0551) ^d	0.1157 (0.0436) ^c	0.1975 (0.0715) ^{cd}	0.0039 (0.0748) ^c
Pt	C	0.4422 (0.0796) ^c	0.1806 (0.0641) ^{cd}	0.1700 (0.1040) ^{cd}	0.2070 (0.1090) ^c

¹ In the case of Ac or Pr to be multiplied by the stoichiometric constant of 2 to obtain the yield of mol Ac or Pr per mol substrate fermented (Equations [2a] & [2b]).

² See Table 1 for explanation of abbreviations, and Section 2 for a more detailed description of the significance tests.

³ Estimates of $Y_{Ac,i}$, $Y_{Pr,i}$, $Y_{Bu,i}$ or $Y_{Bc,i}$ (standard error within parentheses) for either R or C diets without a common superscript significantly differed between distinct types of fermented substrate (*F-test*, $P < 0.05$).

⁴ Significant difference between R diets ($n = 86$) and C diets ($n = 96$) in $Y_{Vfa,i}$ estimate (standard error between parentheses) for a specific combination of type of VFA and type of fermented substrate (*F-test*, ** $P < 0.05$, * $P < 0.1$).

Estimates of in vivo VFA production

Estimates of the stoichiometric coefficients of VFA production from fermented substrates, $Y_{Vfa,i}$, were significantly different for the different types of substrate. Most significant differences were established for Ac and Pr production (Table 5), which indicates that changes in the type and the amount of substrate fermented will have an effect on the production rates of these two VFA in particular. In general, more starch fermentation will increase Pr production, whereas more cellulose fermentation will increase Ac production. The fermentation of Hc, and to a lesser extent that of Sc, appears to stimulate the production of Bu. A high Bc production is frequently assumed on Pt fermentation (Baldwin, 1995). However, in the present study a tendency was found for this with C diets only. Another interesting finding is the different molar proportion of individual VFA, $p_{j,Vfa}$, produced from Ce and Hc. Relatively more Bu and less Ac is produced from fermented Hc than from fermented Ce. Also the type of diet is a relevant regarding the type of VFA produced from distinct substrate types. Again, most significant differences between R and C diets were

established for Ac and Pr production (Table 5). It is interesting to note the higher standard error of the coefficient estimates with C diets than with R diets (up to 150%; Table 5) despite the comparable size of both data sets (86 vs. 96 diets). Apparently, the data set of C diets contained more variation that could not be explained by the model. A direct comparison of the results of cultures of rumen micro-organisms (Russell, 1984; Van Soest, 1994) with the $Y_{Vfa,i}$ values estimated in the present study is complicated by the ability of micro-organisms to ferment a combination of substrate types and by the fact that products of substrate fermented by a certain species of micro-organism, other than VFA (lactate and succinate; Van Soest, 1994), may be fermented further by another species of micro-organism. Although in the present study separate $Y_{Vfa,i}$ were estimated for every substrate type, Sc, St, and Pt in particular are fermented in combination with other substrate types by most rumen microbial species. Hence, it is difficult to make direct comparisons with observations on cultures of micro-organisms and on rumen ecology. But, the estimated $Y_{Vfa,i}$ seem to be in line with reported patterns of VFA for the most important types of micro-organisms and their substrate requirements (Baldwin & Allison, 1983; Russell, 1984; Van Soest, 1994).

Importance of non-represented aspects

The estimated $Y_{Vfa,i}$ for VFA production seem useful to predict VFA production in the rumen of lactating dairy cows. This presumes that, in comparison to the effect of the type of fermented substrate, other factors which are not yet represented had a smaller effect on the type of VFA produced. If future evaluations indicate that the current $Y_{Vfa,i}$ estimates are not of sufficient accuracy, some of these factors will have to be included in the model. A factor worthwhile to include is the fractional rate of VFA absorption in combination with that of rumen fluid. The effect of different fractional absorption rates of VFA could be accounted for if good estimates were available. Estimates may be obtained from empirical equations of VFA absorption (Dijkstra *et al.*, 1993). But, the extent of metabolism of VFA during their absorption through the rumen wall influences the concentration difference of VFA across the epithelial membranes. Thereby, the impact of VFA metabolism on the transport rate of VFA across epithelial membranes cannot be ruled out, which complicates the estimation of the fractional rates of VFA absorption (Bannink *et al.*, 2000). In the present study it was presumed that observed $p_{j,Vfa}$ is identical to the molar proportions in which VFA are produced. If perhaps justified for R diets, for C diets with lower average rumen pH values this is less likely (Dijkstra *et al.*, 1993; Sutton, 1985). The larger standard deviations of $Y_{Vfa,i}$ estimates of C diets in comparison to those of R diets is perhaps the result of a larger variation in VFA absorption rates for C diets. From VFA absorption trials *in vivo* it may be concluded that fractional rates of Ac absorption are lower than that of Pr and Bu (Dijkstra *et al.*, 1993). In this case the assumption of equal fractional absorption rates adopted in the present study would be erroneous and cause the estimated set of $Y_{Vfa,i}$ values to overpredict Ac production and underpredict Pr and Bu production. If this error was larger for C diets than for R diets,

then the differences between R and C diets in estimated $Y_{Vfa,i}$ values established in the present study might have been underestimated. The potential to explain the observed variation in $p_{j,Vfa}$ by regression analysis is relatively insensitive to a systematic (consistent over all diets) error introduced by erroneous assumptions on VFA absorption rates. However, such erroneous assumptions do result in a systematic bias of $Y_{Vfa,i}$ estimates and of the rates of VFA production that are predicted from these $Y_{Vfa,i}$. The sensitivity of $Y_{Vfa,i}$ estimates for the accuracy of VFA absorption rate is illustrated by comparing regression results obtained with identical values for $k_{Abs,j}$ for all VFA (Table 6), with regression results obtained with a value of 3.0 /d for $k_{Out,j}$ for all VFA and values of 6.0, 9.0, 9.0 and 9.0 /d for $k_{Abs,Ac}$, $k_{Abs,Pr}$, $k_{Abs,Bu}$, $k_{Abs,Bc}$, respectively. Adopting the latter values changed the coefficient estimates of Ac production with -13% and -14% for R and C diets, respectively, when averaged over all types of substrate, in contrast to a change of +28% and +16%, +16% and +15%, and +16% and +22% established for Pr, Bu and Bc production, respectively. Notwithstanding these large effects, the differences in coefficient estimates among individual types of VFA, among different types of fermented substrate and among the different estimates for R and C diets, remained remarkably similar to the results shown in Table 6. Besides the fractional rate of VFA absorption, some other factors may be included in the model as well in order to improve the prediction of VFA production. The present model describes an unaltered molar proportion of VFA produced when the increase in fermentation rate is relatively equal for all types of substrate. In reality, however, this molar proportion may vary with the fractional rate of substrate fermentation and the accompanied microbial growth. High rates of substrate fermentation induce a shift in fermentation pathway from the production of Ac to production of Pr, and to a minor extent of Bu, which is thermodynamically more feasible under those conditions (Counotte, 1981; Kohn & Boston, 2000). This shift can be explained from thermodynamical principles and is induced by an accumulation of reduced co-factors. Representing this may become of particular importance when the effects of non-steady state conditions on molar VFA proportions needs to be represented. Inclusion of this aspect in the present model requires that the $Y_{Vfa,i}$ coefficients become functions of fermentation rate, or perhaps of rumen pH, instead of being considered constants. The present approach already accounts for all effects on the rate of rumen substrate digestion because observed values are used as independent model inputs. In this way, for example, high- fermentation rates which lower rumen pH and subsequently limit rumen degradation of neutral detergent fibre (Argyle & Baldwin, 1988) are already taken into account. However, changes in the stoichiometry of VFA produced from fermented substrate, induced by the factors discussed above, are not yet accounted for. Finally, some further aspects may influence microbial metabolism and the molar proportions of VFA produced. Firstly, the partitioning of fermented substrate into substrate converted into VFA and substrate incorporated in microbial mass varies with rumen fermentation conditions. However, its representation would require a much more detailed model of microbial metabolism (e.g. Dijkstra *et al.*, 1992). Secondly, the representation of

more than a single type of fermenting micro-organism may further improve the explanation of VFA molar proportions. The activity of specific types of micro-organism is linked to the type of substrate fermented. Although the latter was represented already in the present model, most species utilize more than one type of substrate and different species of micro-organisms have different substrate requirements and produce a different pattern of VFA (Nagorcka *et al.*, 2000). Notwithstanding this complexity, a logical distinction between amylolytic and cellulolytic micro-organisms seems appropriate. However, this distinction has already been made in the present model because the activity of the former is linked to Sc and St fermentation, and that of the latter by the representation of Hc and Ce fermentation. More useful might be a distinction of the fermentation by protozoa because protozoa utilize all types of substrate, and are known to produce relatively more Bu in comparison to bacteria (Baldwin & Allison, 1983; Counotte, 1981; Czerkawski, 1986; Williams & Coleman, 1988). Hence, changes in the rumen population of protozoa may influence the $p_{j,Vfa}$ found in rumen fluid. As a result, the effect of the type of fermented substrate does not represent completely the effect of the type of fermenting micro-organism. The model used in the present study is capable only to explain variation in $p_{j,Vfa}$ that is related to the type of fermented substrate, and probably covers the effect of changes in the composition of the microbial population to a large extent, but not entirely. In conclusion, further explanation of molar proportions of VFA produced may be achieved when a more detailed rumen model is used in the regression analysis. At least factors like rumen pH and the fractional rates of VFA absorption need to be explored. However, inclusion of additional factors may result in a larger number of parameter values compared to the number used in the present study. It is unlikely that with a substantial increase in the number of parameters, still unique parameter estimates will be derived.

Conclusions

Derived coefficient estimates for VFA production from fermented substrate in the rumen of lactating cows significantly differ from those established previously. Only a rather small fraction of the observed variation in VFA molar proportions in rumen fluid can be explained by the statistical approach applied. Similar results are obtained when regressing against simulated data of VFA molar proportions, which demonstrates that this outcome is inevitable with the this type of data and type of statistical analysis. Such a limited explanation was not established for rates of VFA production and the use of such observational data is therefore clearly to be preferred. This new set of coefficient estimates may significantly improve the prediction of VFA production by extant models of whole rumen function. The value of the current estimates needs to be evaluated and compared to results of Murphy *et al.* (1982), and the more recently published results of Friggens *et al.* (1998) and Pitt *et al.* (1996). When applying these estimates, consideration needs to be given to the assumptions and the concepts used in the models the coefficient estimates were derived with.

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References

- Aldrich, J.M., Muller, L.D., Varga, G.A. & Griel Jr., L.C., 1993. Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. *Journal of Dairy Science* 76, 1091-1105.
- Argyle, J.L. & Baldwin, R.L., 1988. Modeling of rumen water kinetics and effects of rumen pH changes. *Journal of Dairy Science* 71, 1178-1188.
- Baldwin, R.L. & Allison, M.J., 1983. Rumen metabolism. *Journal of Animal Science* 57 (supplement 2), 461-477.
- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating dairy cow. II. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54, 107-131.
- Baldwin, R.L., 1995. *Modeling Ruminant Digestion and Metabolism*. Chapman & Hall, London, United Kingdom.
- Bannink, A., De Visser, H., Van Vuuren, A.M., 1997a. Comparison and evaluation of mechanistic rumen models. *British Journal of Nutrition* 78, 563-581.
- Bannink, A., De Visser, H., Klop, A., Dijkstra, J. & France, J., 1997b. Causes of inaccurate prediction of volatile fatty acids by simulation models of rumen function in lactating cows. *Journal of Theoretical Biology* 89, 353-366.
- Bannink, A., Kogut, J., Dijkstra, J., France, J., Tamminga, S., Van Vuuren, A.M., 2000. Modelling production and portal appearance of volatile fatty acids in dairy cows. In: J.P. McNamara, J. France & D.E. Beever (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 87-102.
- Beever, D.E., Sutton, J.D., Thomson, D.J., Napper, D.J. & Gale, D.L., 1988. Comparison of dried molassed and unmolassed sugar beet feed and barley as energy supplements on nutrient digestion and supply in silage fed cows. British Society of Animal Production, Winter Meeting 1988.
- Benchaar, C., Bayourthe, C., Moncoulon, R., Vernay, M., 1991. Digestion ruminale et absorption intestinale des protéines du lupin extrudé chez la vache laitière. *Reproduction Nutrition Development* 31, 655-665.
- Bibby, J. & Toutenburg, H., 1977. *Prediction and Improved Estimation in Linear Models*. Wiley, London, UK.

- Calsamiglia, S., Caja, G., Stern, M.D., Crooker, B.A., 1995. Effects of ruminal versus duodenal dosing of fish meal on ruminal fermentation and milk composition. *Journal of Dairy Science* 78, 1999-2007.
- Cameron, M.R., Klusmeyer, T.H., Lynch, G.L., Clark, J.H., Nelson, D.R., 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. *Journal of Dairy Science* 74, 1321-1336.
- Christensen, R.A., Cameron, M.R., Klusmeyer, T.H., Elliott, J.P., Clark, J.H., Nelson, D.R., Yu, Y., 1993. Influence of amount and degradability of dietary protein on nitrogen utilization by dairy cows. *Journal of Dairy Science* 76, 3497-3513.
- Counotte, G.H.M., 1981. *Regulation of lactate metabolism in the rumen*. PhD Thesis, University of Utrecht, Utrecht, The Netherlands.
- Cunningham, K.D., Cecava, M.J. & Johnson, T.R., 1993. Nutrient digestion, nitrogen, and amino acid flows in lactating cows fed soybean hulls in place of forage or concentrate. *Journal of Dairy Science* 76, 3523-3535.
- Cunningham, K.D., Cecava, M.J., Johnson, T.R., 1994. Flows of nitrogen and amino acids in dairy cows fed diets containing supplemental feather meal and blood meal. *Journal of Dairy Science* 77, 3666-3675.
- Cunningham, K.D., Cecava, M.J., Johnson, T.R. & Ludden, P.A., 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. *Journal of Dairy Science* 79, 620-630.
- Czerkawski, J.W., 1986. *An Introduction to Rumen Studies*. Pergamon Press, Oxford, United Kingdom.
- De Visser, H., Huisert, H., Ketelaar, R.S., 1991. Dried beet pulp, pressed beet pulp and maize silage as substitutes for concentrates in dairy cow rations. 2. Feed intake, fermentation pattern and ruminal degradation characteristics. *Netherlands Journal of Agricultural Science* 39, 21-30.
- De Visser, H., Huisert, H., Klop, A., Ketelaar, R.S., 1993. Autumn-cut grass silage as roughage component in dairy cow rations. 2. Rumen degradation, fermentation and kinetics. *Netherlands Journal of Agricultural Science* 41, 221-234.
- De Visser, H., Valk, H., Klop, A., Van Der Meulen, J., Bakker, J.G.M., Huntington, G.B., 1997. Nutrient fluxes in splanchnic tissue of dairy cows: influence of grass quality. *Journal of Dairy Science* 80, 1666-1673.
- Dijkstra, J., Neal, H.D.StC., Beever, D.E. & France, J., 1992. Simulation of nutrient digestion, absorption, and outflow in the rumen: Model description. *Journal of Nutrition* 122, 2239-2256.
- Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M. & Tamminga, S., 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *British Journal of Nutrition* 69, 385-396.

- Dijkstra, J. & Bannink, A., 2000. Analyses of modelling whole-rumen function. In: M.K. Theodorou & J. France (Eds.), *Feeding Systems and Feed Evaluation Models*. CAB International, Wallingford, United Kingdom, pp. 299-322.
- Erasmus, L.J., Botha, P.M. & Meissner, H.H., 1994. Effect of protein source on ruminal fermentation and passage of amino acids to the small intestine of lactating cows. *Journal of Dairy Science* 77, 3655-3665.
- Feng, P., Hoover, W.H., Miller, T.K. & Blauwiekel, R., 1993. Interactions of fiber and nonstructural carbohydrates on lactation and ruminal function. *Journal of Dairy Science* 76, 1324-1333.
- Ferlay, A., Legay, F., Bauchart, D., Poncet, C. & Doreau, M., 1992. Effect on a supply of raw or extruded rapeseeds on digestion in dairy cows. *Journal of Animal Science* 70, 915-923.
- Friggens, N.C., Oldham, J.D., Dewhurst, R.J., Horgan, G., 1998. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage. *Journal of Dairy Science* 81, 1331-1344.
- GENSTAT, 1993. *Genstat 5 Release 3 Reference Manual*. Clarendon Press, Oxford, United Kingdom.
- Haïmoud, D.A., Vernay, M., Bayourthe, C. & Moncoulon, R., 1995. Avoparcin and monensin effects on the digestion of nutrients in dairy cows fed a mixed diet. *Canadian Journal of Animal Science* 75, 379-385.
- Herrera-Saldana, R., Gomez-Alarcon, R., Torabi, M., Huber, J.T., 1990. Influence of synchronizing protein and starch degradation in the rumen of nutrient utilization and microbial protein synthesis. *Journal of Dairy Science* 73, 142-148.
- Hespell, R.B., Bryant, M.P., 1979. Efficiency of rumen microbial growth: influence of some theoretical and experimental factors on YATP. *Journal of Animal Science* 49, 1640-1659.
- Khorasani, G.R., Okine, E.K., Kanelly, J.J., 1996. Forage source alters nutrient supply to the intestine without influencing milk yield. *Journal of Dairy Science* 79, 862-872.
- Klop, A. & De Visser, H., 1994. The effect of different grass silage: Maize silage ratios in the ration on feed intake, milk production, rumen fermentation, rumen kinetics and faecal digestibility. ID-DLO (IVVO) Report 270, 31pp.
- Klusmeyer, T.H., McCarthy Jr., R.D. & Clark, J.H., 1990. Effects on source and amount of protein on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. *Journal of Dairy Science* 73, 3526-3537.
- Klusmeyer, T.H., Lynch, G.L., Clark, J.H., Nelson, D.R., 1991a. Effects of calcium salts of fatty acids and protein source on ruminal fermentation and nutrient flow to duodenum of cows. *Journal of Dairy Science* 74, 2206-2219.

- Klusmeyer, T.H., Lynch, G.L., Clark, J.H., Nelson, D.R., 1991b. Effects of calcium salts of fatty acids and proportion of forage in diet on ruminal fermentation and nutrient flow to duodenum of cows. *Journal of Dairy Science* 74, 2220-2232.
- Kohn, R.A. & Boston, R.C., 2000. The role of thermodynamics in controlling rumen metabolism. In: J.P. McNamara, J. France & D.E. Beever (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, UK, pp. 11-24.
- Koong, L.J., Baldwin, R.L., Ulyatt, M.J., Charlesworth, T.J., 1975. Iterative computation of metabolic flux and stoichiometric parameters for alternate pathways in rumen fermentation. *Computer Programs in Biomedicine* 4, 209-213.
- Kung Jr., L., Huber, J.T. & Satter, L.D., 1983. Influence of nonprotein nitrogen and protein of low rumen degradability on nitrogen flow and utilization in lactating dairy cows. *Journal of Dairy Science* 66, 1863-1872.
- Lynch, G.L., Klusmeyer, T.H., Cameron, M.R., Clark, J.H. & Nelson, D.R., 1991. Effects of somatotropin and duodenal infusion of amino acids on nutrient passage to duodenum and performance of dairy cows. *Journal of Dairy Science* 74, 3117-3127.
- Mansfield, H.R. & Stern, M.D., 1994. Effects of soybean hulls and lignosulfonate-treated soybean meal on ruminal fermentation in lactating dairy cows. *Journal of Dairy Science* 77, 1070-1083.
- McCarthy, R.D., Klusmeyer, T.H., Vicini, J.L., Clark, J.H. & Nelson, D.R., 1989. Effects on source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. *Journal of Dairy Science* 72, 2002-2016.
- McNiven, M.A., Weisbjerg, M.R. & Hvelplund, T., 1995. Influence of roasting or sodium hydroxide treatment of barley on digestion in lactating cows. *Journal of Dairy Science* 78, 1106-1115.
- Murphy, M.R., Baldwin, R.L. & Koong, L.J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *Journal of Animal Science* 55, 279-296.
- Nagorcka, B.N., Gordon, G.L.R. & Dynes, R.A., 2000. Towards a more accurate representation of fermentation in mathematical models of the rumen. In: J.P. McNamara, J. France & D.E. Beever (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 37-48.
- Neal, H.D.StC., Dijkstra, J. & Gill, M., 1992. Simulation of nutrient digestion, absorption, and outflow in the rumen: Model evaluation. *Journal of Nutrition* 122, 2257-2272.
- Overton, T.R., Cameron, M.R., Elliott, J.P., Clark, J.H. & Nelson, D.R., 1995. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. *Journal of Dairy Science* 78, 1981-1998.
- Palmquist, D.L., Weisbjerg, M.R. & Hvelplund, T., 1993. Ruminal, intestinal, and total digestibilities of nutrients in cows fed diets high in fat and undegradable protein. *Journal of Dairy Science* 76, 1353-1364.

- Pantoja, J., Firkins, J.L. & Eastridge, M.L., 1995. Site of digestion and milk production by cows fed fats differing in saturation, esterification, and chain length. *Journal of Dairy Science* 78, 2247-2258.
- Pena, F., Tagari, H. & Satter, L.D., 1986. The effect of heat treatment of whole cottonseed on site and extent of protein digestion in dairy cows. *Journal of Animal Science* 62, 1423-1433.
- Pitt, R.E., Van Kessel, J.S., Fox, D.G., Pell, A.N., Barry, M.C. & Van Soest, P.J., 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. *Journal of Animal Science* 74, 226-244.
- Poore, M.H., Moore, J.A., Swingle, R.S., Eck, T.P. & Brown, W.H., 1993. Response of lactating holstein cows to diets varying in fiber source and ruminal starch degradability. *Journal of Dairy Science* 76, 2235-2243.
- Robinson, P.H., Tamminga, S. & Van Vuuren, A.M., 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.
- Robinson, P.H. & Kennelly, J.J., 1990. Evaluation of duodenal cannula for dairy cattle. *Journal of Dairy Science* 73, 3146-3157.
- Robinson, P.H., De Boer, G. & Kennelly, J.J., 1991. Effect of bovine somatotropin and protein on rumen fermentation and forestomach and whole tract digestion in dairy cows. *Journal of Dairy Science* 74, 3505-3517.
- Rohr, K., Schafft, H. & Honing, H., 1986. Zum Einfluss einer intensiven nachzerkleinerung von maissilage auf die stoffumsetzungen in den vormagen der milchkuhe. *Journal of Animal Physiology and Animal Nutrition* 55, 121-128.
- Russell, J.B., 1984. Factors influencing competition and composition of the rumen bacterial flora. In: F.C.M. Gilchrist & R.I. Mackie (Eds.), *Proceedings of the International Symposium on Herbivore Nutrition in the Subtropics and Tropics*. The Science Press, Craighall, South Africa, pp. 313-345.
- Santos, K.A., Stern, M.D. & Satter, L.D., 1984. Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. *Journal of Animal Science* 58, 244-255.
- Stern, M.D., Rode, L.M., Prange, R.W., Stauffacher, R.H. & Satter, L.D., 1983. Ruminal protein degradation of corn gluten meal in lactating dairy cattle fitted with duodenal t-type cannulae. *Journal of Animal Science* 56, 194-205.
- Stokes, S.R., Hoover, W.H., Miller, T.K. & Blauweikel, R., 1991. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. *Journal of Dairy Science* 74, 871-881.
- St-Pierre, N.R., 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. *Journal of Dairy Science* 84, 741-755.

- Strobel, H.J. & Russell, J.B., 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. *Journal of Dairy Science* 69, 2941-2947.
- Sutton, J.D., Oldham, J.D. & Hart, I.C., 1980. Products of digestion, hormones and energy utilization in milking cows given concentrates containing varying proportions of barley and maize. In: L.E. Mount (Ed.), *Energy Metabolism*, Butterworths, London, United Kingdom, pp. 303-306.
- Sutton, J.D., 1985. Digestion and absorption of energy substrates in the lactating cow. *Journal of Dairy Science* 68, 3376-3393.
- Tamminga, S., Van Vuuren, A.M., Van Der Koelen, C.J., Khattab, H.M., Van Gils, L.G.M., 1983. Further studies on the effect of fat supplementation of concentrates fed to lactating dairy cows. 3. Effect on rumen fermentation and site of digestion of dietary components. *Netherlands Journal of Agricultural Science* 31, 249-258.
- Tice, E.M., Eastridge, M.L. & Firkins, J.L., 1993. Raw Soybeans and roasted soybeans of different particle sizes, 1. digestibility and utilization by lactating cows. *Journal of Dairy Science* 76, 224-235.
- Van Soest, P.J., 1994. Microbes in the gut. In: P.J. Van Soest (Ed.), *Nutritional Ecology of the Ruminant*. O&B Books, Corvallis, United States of America, pp. 257-280.
- Van Vuuren, A.M., Van Der Koelen, C.J., Valk, H. & De Visser, H., 1993a. Effects of partial replacement of ryegrass by low protein feeds on rumen fermentation and nitrogen loss by dairy cows. *Journal of Dairy Science* 76, 2982-2993.
- Van Vuuren, A.M., Van Der Koelen, C.J., Vroons De Bruin, J., 1993b. Ryegrass versus corn starch or beet pulp fiber diet effects on digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science* 76, 2692-2700.
- Waltz, D.M., Stern, M.D. & Illg, D.J., 1989. Effect of ruminal protein degradation of blood meal and feather meal on the intestinal amino acid supply to lactating cows. *Journal of Dairy Science* 72, 1509-1518.
- Windschitl, P.M. & Stern, M.D., 1988. Evaluation of calcium lignosulfonate-treated soybean meal as a source of rumen protected protein for dairy cattle. *Journal of Dairy Science* 71, 3310-3322.
- Williams, A.G. & Coleman, G.S., 1988. The rumen protozoa. In: P.N. Hobson (Ed.), *The Rumen Microbial Ecosystem*. Elsevier Science Publishers, London, United Kingdom, pp. 77-128.
- Zerbini, E., Polan, C.E. & Herbein, J.H., 1988. Effect of dietary soybean meal and fish meal on protein digesta flow in holstein cows during early and midlactation. *Journal of Dairy Science* 71, 1248-1258.

Chapter 7

Modelling the Implications of Feeding Strategy on Rumen Fermentation and Functioning of the Rumen Wall

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Abstract

The present study gives a critique of the mechanisms involved with the formation of volatile fatty acid (VFA) formed in the lumen of the reticulo-rumen, the absorption of VFA across the reticulorumen wall, and the intra-epithelial metabolism of VFA by reticulo-rumen epithelium. In contrast to the empirical treatment of these aspects in previous rumen modelling studies, a mechanistic model was developed which represents each of these aspects separately. Because tissues of the reticulo-rumen may strongly adapt to changing nutritional conditions, this adaptive response was included in the model. The model enabled an evaluation of the implications of VFA yield on the development of the rumen wall, on the transport of VFA, on the extent of intra-epithelial metabolism of VFA, and on the consequences for the supply of VFA to the ruminant. The current modelling effort allowed the integration of existing knowledge on each of these aspects and the model reproduced some essential characteristics of experimental observations on VFA absorption and metabolism. Although further development is still needed, the model appears helpful to distinguish elements that require specific consideration when evaluating rates of net portal appearance of VFA, or when testing hypothesis on the interaction between formation, absorption and intra-epithelial metabolism of VFA under various experimental conditions.

Abbreviations: Ac, acetate; Bu, butyrate; dVFA, dissociated VFA; VF_{Ai}, intra-epithelial VFA; Pr, propionate; uVFA, undissociated VFA; VFA, volatile fatty acids

Introduction

Published models of the dynamics of rumen function (*e.g.* Baldwin *et al.*, 1987; Dijkstra *et al.*, 1992; Pitt *et al.*, 1996) represent the fermentative processes that take place in the lumen in particular. The aspects represented are usually the dynamics of microbial activity, of passage of particulate and soluble matter and of absorption by the rumen wall. Typical outcomes of these models are the digestibility of distinct chemical fractions, and yields of microbial mass, volatile fatty acids (VFA), ammonia and gases as end-products of fermented substrate. The integrated nature of these models makes them useful to explain observed relationships between nutrition and rumen function (Dijkstra *et al.*, 2002) and to

evaluate nutrient partition between the animal and the environment (Kebreab *et al.*, 2004). Furthermore, these models are suitable for predicting the type of nutrient supplied to the ruminant and consequently required in whole animal models (Baldwin, 1995).

As part of the visceral organs drained by the portal vein, tissues of the rumen wall have a high metabolic activity which appears to be related to the high microbial activity in the lumen and the load of VFA transported by them (Lobley *et al.*, 1994; Lindsay & Reynolds, 2005). Digesta load, VFA production rate and type of VFA strongly affect the development and proliferation of these tissues in the rumen wall. In calves, development of rumen mucosa is affected positively by VFA produced in the rumen, but negatively by rumen lactate (Suàrez *et al.*, 2006). Relatively little information is available, however, on the impact of tissue state and activity on rumen function and modelling efforts in this area seem to be lacking. Extant dynamic rumen models do consider the process of VFA absorption by the rumen wall, but relate this to characteristics of rumen contents and not to the physiology of the rumen wall. Further, a major part of the energy requirement by tissues in the rumen wall is met by the metabolism of VFA transported from lumen to blood. Hence, it seems likely that there is interdependence between rumen fermentation processes and the metabolic activity and functioning of the rumen tissues, and that both are important determinants of rumen function.

The objective of the present study was to study by a modelling approach the effect of rumen VFA yield and adaptation of the rumen wall on the transport of VFA across the rumen wall and the intra-epithelial VFA metabolism. Concepts of adaptation of the rumen wall were based on recent observations on the effect of feeding strategy on development of the rumen wall in dairy cows during early lactation (Bannink *et al.*, 2005).

Rumen function

When representing rumen function, numerous aspects need to be integrated: comminution of feed particles, degradation of substrates, microbial metabolism and growth, formation of end-products of fermentation (VFA, ammonia, gases), passage of substrates and of microbial matter and other end-products of fermentation, absorption of VFA and ammonia, and recycling of specific compounds from blood to rumen (urea, saliva, water). Representations of rumen wall functioning normally refer to the capacity to absorb VFA and ammonia and to the quantity of N recycled to the rumen from blood. Below, the representation adopted in most models of rumen function will be discussed, together with a discussion of the dynamics of VFA yield, VFA absorption, VFA metabolism and physiology of rumen tissues. With respect to the dynamic modelling of microbial fermentation in the rumen, the reader is referred to the numerous reviews that have already been published (Baldwin, 1995; Bannink & De Visser, 1997; Dijkstra *et al.*, 2002; Offner & Sauvant, 2004).

VFA yield

Biology

Micro-organisms utilize substrates for purposes of biosynthesis and for the generation of metabolic energy and reducing potential. For the latter, substrates are fermented to VFA and ammonia. It is generally assumed that changes in the type of VFA formed are related to three interdependent factors. Firstly, the type of VFA formed depends on the composition of microbial population in the rumen. Because each microbial species shows substrate specificity and characteristic metabolic pathways (giving rise to a distinct pattern of VFA produced), a change in microbial population in the rumen as a whole may also cause a change in the type of VFA formed in the rumen. With different feeding and intra-ruminal conditions, the composition of the microbial population may also differ because of the niche that each particular species occupies in the rumen environment. Secondly, the type of VFA formed is related to the type of substrate fermented. Because the type of fermenting micro-organism will change together with a change in type of substrate fermented, both factors are strongly interrelated. Thirdly, the type of VFA formed depends on the changes in environmental conditions within the rumen or the characteristics of microbial metabolism. A higher rate of fermentation and faster microbial growth may cause shifts in the abundance of microbial species as well as in the type of VFA formed by a species. For this reason, with identical organic matter fermented increased proportions of propionate in particular are observed *in vivo* as well as *in vitro* if fermentation rates increase. The thermodynamical principles of this shift have been explained by Kohn & Boston (2000).

Modelling approaches

Several modelling approaches have been presented to represent the effect of the above three factors on the type of VFA formed. Empirical approaches used by Friggens *et al.* (1998), Lescoat & Sauvant (1995) and Hanigan (2005) relate the molar proportion of a specific type of VFA (f_{VFA}) in the total of VFA formed to general nutritional factors such as the dietary content of fibre, starch and protein, or the forage to concentrate ratio (Lescoat & Sauvant, 1995). However, molar proportions of VFA are not just related to type of substrate fermented but to other factors as well.

Another empirical approach with direct observations of patterns of VFA in rumen fluid related to observed amounts of rumen digested substrate, but with more mechanistic elements included (representation of microbial metabolism), was developed by Murphy *et al.* (1982) and adapted recently by Bannink *et al.* (2000, 2006a). On first appearance, similar factors have been included in the regression analysis to those used with the more empirical approaches. An important distinction is, however, that direct observations were used of the fermentation conditions actually met in the rumen environment, instead of making use of general and much less specific factors such as dietary contents. Bannink *et al.* (2000, 2006a)

derived a new set of stoichiometric coefficients for VFA production specific for high-yielding lactating dairy cows by making use of observations of rumen fermentation for this type of animal only (in contrast to Murphy *et al.*, 1982; Table 1). Although the broader applicability of these new coefficient values has been questioned (Hanigan, 2005) the poor prediction of VFA molar proportions in the rumen of lactating cows using the Murphy approach (Neal *et al.*, 1992; Bannink *et al.*, 1997) seems to have been improved (Mills *et al.*, 2001; Bannink & Tamminga, 2005). Other factors of interest may be included in the analysis of fVFA. For example, Argyle & Baldwin (1988) introduced the effect of rumen pH on the fVFA for soluble carbohydrates and starch as fermented substrates, based on *in vitro* results. Recently, Bannink *et al.* (2006b) elaborated on the model of VFA formation by Bannink *et al.* (2006a) by including the effect of rumen pH while taking into account the effects of rumen fluid volume, fractional rates of fluid passage and different fractional absorption rates for individual VFA on VFA molar proportions in rumen fluid. Sveinbjörnsson *et al.* (2006) elaborated on the work by Bannink *et al.* (2000) by introducing additional substrate classes (lactate and a distinction between NDF of roughage and of concentrate origin) and additional coefficients to correct for the effect of feed intake level and concentrate ether extract on VFA formation.

Table 1. Comparison of coefficients derived by Murphy *et al.* (1982; M) and Bannink *et al.* (2006a; B) for the fraction of fermented substrate converted into volatile fatty acids

	Diet type	Ac		Pr		Bu		Bc	
		M	B	M	B	M	B	M	B
Soluble carbohydrates	R	0.69	0.64	0.21	0.08	0.10	0.24	0.00	0.04
Starch	R	0.60	0.49	0.14	0.22	0.20	0.21	0.06	0.08
Hemi-cellulose	R	0.57	0.44	0.18	0.18	0.21	0.32	0.05	0.06
Cellulose	R	0.66	0.56	0.09	0.20	0.23	0.17	0.03	0.07
Protein	R	0.08 ¹	0.56	0.07	0.29	0.08	0.08	0.03 ¹	0.06
Soluble carbohydrates	C	0.45	0.53	0.21	0.16	0.30	0.26	0.04	0.06
Starch	C	0.40	0.49	0.30	0.31	0.20	0.15	0.10	0.05
Hemi-cellulose	C	0.56	0.51	0.26	0.12	0.11	0.32	0.07	0.05
Cellulose	C	0.79	0.68	0.06	0.12	0.06	0.20	0.09	0.00
Protein	C	0.08 ¹	0.44	0.08	0.18	0.08	0.17	0.03 ¹	0.21

Ac: acetate; Pr: propionate; Bu: butyrate; Bc: valerate and branched chain volatile fatty acids; R: roughage-rich diets; C: concentrate-rich diets.

¹ Bannink *et al.* (2006a) derived coefficients for the partition among individual VFA formed from pyruvate units that originate from fermented protein. Murphy *et al.* (1982) derived similar coefficients but with the assumption of a constant amount of 0.21 mol Ac and 0.30 mol Bc/mol fermented protein. These amounts of Ac and Bc need to be added to the amounts calculated using the table coefficient values.

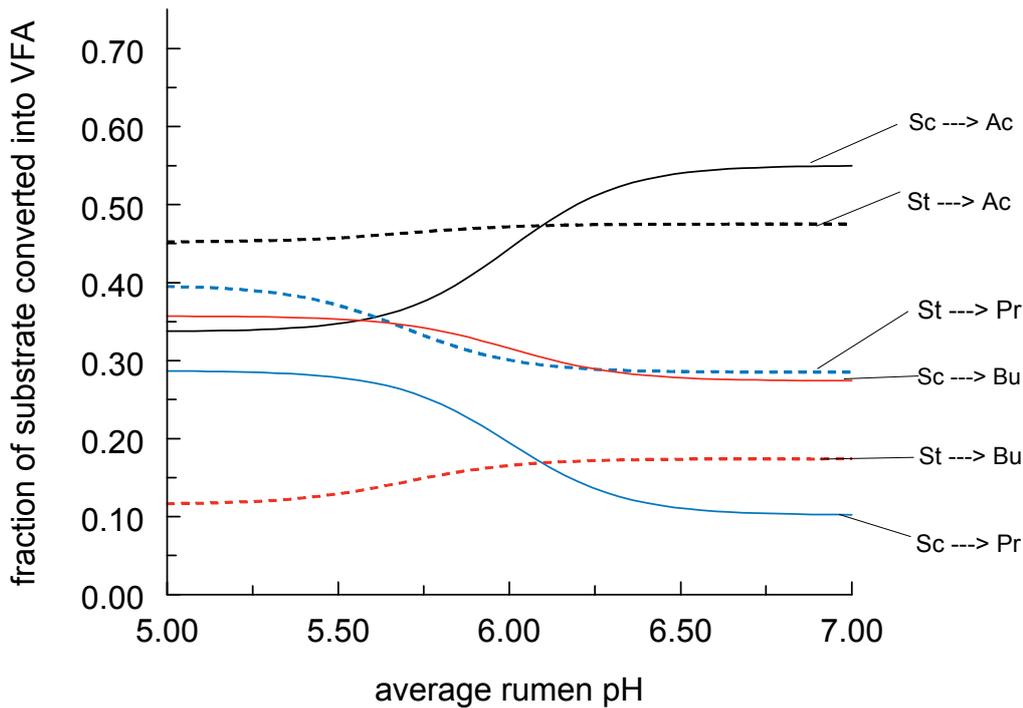


Figure 1. Fitted effect of rumen pH on the fraction of the individual types of VFA (Ac, Pr, Bu: acetate, propionate, butyrate, respectively) produced from rapidly fermentable carbohydrates converted to VFA in concentrate-rich diets (Sc: soluble carbohydrates including the fraction remaining undetermined with feed analysis; St: starch; according to Bannink *et al.*, 2006b).

Instead of using observations of rumen substrate digestion, more general characteristics were used in the regressions study however (contents of fermentable substrates and of concentrate ether extract in dietary dry matter, and feed intake per kilogram of live weight). As a result, the approach can be considered intermediary between the empirical approaches mentioned initially and the more mechanistic approaches of Murphy *et al.* (1982) and Bannink *et al.* (2006a, b). With the latter, regressions results were based fully on observations of the actual rumen conditions met during the experiment rather than on general dietary characteristics and level of feed intake.

To include the effect of pH on VFA yields from soluble carbohydrates and starch, best results were obtained from *in vivo* rumen observations in lactating cows when making fVFA dependent on pH by applying symmetrical logistic equations (Bannink *et al.*, 2006b). The type of relationships obtained is presented in Figure 1. With *in vivo* data, no satisfactory regression results were obtained when applying the linear equations proposed by Argyle and Baldwin (1988), who based their equations on *in vitro* results. An improvement of the representation of factors that determine f_{VFA} is still needed, however, and there is a need for direct measurement of the rate of VFA production *in vivo* by the use of stable isotopes techniques.

VFA clearance

Biology

It has been clearly demonstrated by *in vivo* VFA clearance trials with lactating cows (Dijkstra *et al.*, 1993) and sheep (López *et al.*, 2003) that rate of VFA clearance depends mostly on rumen VFA concentration. Factors influencing VFA clearance rate are pH, volume of rumen fluid (indicative of absorptive area) and fluid outflow rate (washout of the rumen rather than absorption across the rumen wall). The latter depends on the water dynamics of the rumen (fluid inflow with saliva, water flow through the rumen wall, and outflow of rumen fluid). The kinetics of VFA clearance appear to differ among individual types of VFA and most absorption trials clearly indicate an increase in fractional absorption rate with an increase in lipophilicity (partition coefficient water/hydrophobic solvent; Baláž, 2000) which is related to the chain length of VFA (Dijkstra *et al.*, 1993; López *et al.*, 2003). Sutton *et al.* (2003) established contrasting results, however, with a lower fractional absorption rate of butyrate (Bu) compared to acetate (Ac) and propionate (Pr) after infusing ¹⁴C-labeled VFA to determine net VFA production rates and to compare these with VFA molar proportions in rumen fluid. A possible explanation may be the relatively high rumen pH of 6.5 and 6.8 on the high-roughage and low-roughage diet, respectively. At similar pH, Dijkstra *et al.* (1993) also reported a non-significantly lower fractional absorption rate for Bu than for Pr but not for Ac. In sheep rumen at pH 6.8, López *et al.* (2003) did not reproduce these results however. In contrast to results with high rumen pH, results at low pH invariably indicate that the fractional absorption rate increases in the order of Ac, Pr and Bu. The lower rumen pH, the more VFA transport is the result of passive diffusion of VFA with rumen concentrations of VFA as the determinant, and the less VFA transport is affected by VFA metabolism and competitive inhibition between individual VFA. Besides absorption across the rumen wall, the outflow of rumen fluid also contributes to clearance of VFA from rumen fluid. Increased osmolality of rumen fluid by salts or higher VFA concentration (Dijkstra *et al.*, 1993; López *et al.*, 2003) increases the volume of rumen fluid and the fractional rate of outflow of fluid and of the VFA contained. Consequently, increased VFA production rate not only stimulates VFA clearance by increased VFA concentration and VFA absorption rate, but also by increased outflow with rumen fluid. This implies that rumen fluid dynamics are an important aspect when considering buffering of rumen contents and functioning of the rumen wall as a response to feeding strategies.

Modelling approaches

Dynamic models of rumen function distinguish the effect of nutrition on outflow of particulate matter from outflow of fluid. In a few models, equations have been added to represent the dynamics of fluid volume (Chilibroste *et al.*, 2001) or the dynamics of inflow and outflow of water (Argyle & Baldwin, 1988). In all models, clearance of VFA with fluid

outflow is represented by mass action flow. For VFA absorption, however, different representations have been used varying from constant and identical values for individual types of VFA (Baldwin *et al.*, 1987), constant and differentiated values for individual types of VFA and dissociated and undissociated VFA (Pitt *et al.*, 1996), to nonlinear equations differentiated for individual types of VFA that describe saturation of absorption with increasing rumen VFA concentrations (Dijkstra *et al.*, 1993). With the latter approach, observed effects of fluid volume, fractional fluid outflow, rumen pH and rumen VFA concentrations on VFA absorption have been combined in a single equation. Dijkstra *et al.* (1993) derived the following equation to represent VFA absorption by the rumen wall:

$$U_{VFA} = \frac{v_{\max,Ab1} Vol^{0.75}}{(1 + (M1/C_{VFA})^{p1}) (1 + (pH/J)^{p2})} \quad [1]$$

where U_{VFA} is VFA absorption rate (mol/d), $v_{\max,Ab1}$ the maximum absorption rate (mol/(l d)), Vol (L) the rumen fluid volume as a representation of absorptive area, $M1$ the affinity constant for VFA absorption (mol/l), C_{VFA} the rumen VFA concentration (mol/l), pH the rumen acidity, J the inhibition constant for VFA absorption (pH unit), and $p1$ and $p2$ are sigmoidal steepness parameters.

Parameterisation of this equation has been described in detail by Dijkstra *et al.* (1993). For the purpose of this study, and following Dijkstra *et al.* (1992), this equation is simplified to

$$U_{VFA} = \frac{v_{\max,Ab2} Vol^{0.75}}{(1 + M2/C_{VFA}) (1 + (pH/J)^{p3})} \quad [2]$$

where $v_{\max,Ab2}$, $M2$ and $p3$ have different values from $v_{\max,Ab1}$, $M1$ and $p2$, respectively.

Two distinct routes of VFA absorption are combined in Equations [1] & [2]. Firstly, there is the route of facilitated (enzymatically driven) transport of dissociated VFA anions (dVFA), which requires energy and saturates with increasing VFA concentration. Secondly, there is a route of passive diffusion of undissociated VFA (uVFA) through the rumen wall from lumen to blood. Rumen concentrations of uVFA and dVFA can be calculated from C_{VFA} and pH according to the Henderson–Hasselbalch equation (Pitt *et al.*, 1996). From Equation [2] (with $J = 6.45$ and $p3 = 6.48$; Dijkstra *et al.*, 1992), Equation [3] can be derived at pH 4 with only 0.13 of the VFA in the dVFA form according to the Henderson–Hasselbalch equation (pKa value of 4.8). This situation therefore excludes most facilitated transport of dVFA (enzymatically driven VFA transport). Equation [4] can be derived for pH 8 with almost no VFA present in the uVFA form and therefore excluding passive diffusion of uVFA:

$$U_{uVFA} = \frac{v_{\max,Ab2} Vol^{0.75}}{(1 + M2/C_{uVFA})^{1.0}} \quad [3]$$

$$U_{dVFA} = \frac{v_{\max,Ab2} Vol^{0.75}}{(1 + M2/C_{dVFA})^{5.0}} \quad [4]$$

where

$$C_{VFA} = C_{uVFA} + C_{dVFA} \quad [5]$$

These equations hence imply that transport of both uVFA and dVFA saturates with increasing concentration. By applying a single regression equation, Dijkstra *et al.* (1992) used a description of the fractional absorption rate of VFA that combines the effect of VFA concentration, pH and fluid volume. The equation is thereby an empirical representation of the combination of physiological factors that affect VFA absorption. These factors not only involve VFA concentrations and pH, but also the VFA concentration gradients between rumen fluid, the intra-epithelial pool and arterial blood, and intra-epithelial metabolism of VFA. Observed saturation of the (fractional) absorption rate hence may be the result of a saturation of transport rate as well as a reduction of VFA concentration gradients. With the aim of studying the interactions between the supply of uVFA and dVFA, a distinction is needed between the facilitated transport of dVFA, the passive diffusion of uVFA, the intra-epithelial metabolism of VFA (uVFA and dVFA), and the concentration gradient of dVFA and uVFA. This means that instead of using the single equation of Dijkstra *et al.* (1992 & 1993) each of these aspects needs to be represented separately. Despite the importance of rumen pH for VFA clearance, it is an input parameter to the current model. The predictability of rumen pH is low (Pitt *et al.*, 1996; Kolver & de Veth, 2002) and does not only depend on rumen VFA concentrations but also on factors such as saliva production and the composition of dietary salts.

Passive diffusion of uVFA. In contrast to Equation [3], the process of passive diffusion of uVFA may be expected to be linearly related to uVFA concentration according to the following equation:

$$U_{uVFA} = k_{uVFA} Vol^{0.75} (C_{uVFA} - C_{uVFAi}) \quad [6]$$

where k_{uVFA} is the fractional rate constant of passive diffusion (distinct values for individual types of VFA related to lipophilicity (l/d.l)), C_{uVFA} and C_{uVFAi} are the rumen and intra-epithelial concentrations of uVFA (mol/l), and

$$C_{\text{VFai}} = C_{\text{uVFai}} + C_{\text{dVFai}} \quad [7]$$

Because C_{uVFai} values will be very low at physiological pH with pKa values of VFA around 4.8, Equation [6] reduces to

$$U_{\text{uVFA}} = k_{\text{uVFA}} \text{Vol}^{0.75} C_{\text{uVFA}} \quad [8]$$

According to the equations of Dijkstra *et al.* (1993), at pH 4, $\text{Vol}=75$ L and $C_{\text{uVFA}} = 0.05$ mol/l, the values of k_{uVFA} would be 24, 48 and 60 l/d l for Ac, Pr and Bu, respectively (ratio of 1.0 : 2.0 : 2.5). Results of López *et al.* (2003) in sheep indicate less difference between individual types of VFA but values were obtained at a pH of 0.4 units higher than pKa of VFA (ratio of 1.0 : 1.4 : 2.1 for Ac : Pr : Bu).

Facilitated transport of dVFA. If VFA accumulate in blood, the VFA concentration gradient and fractional rate of VFA transport from lumen to blood decrease. However, this is not a very likely explanation of the lower fractional absorption rate of Bu established by Sutton *et al.* (2003), because normally arterial Bu concentrations remain low (Reynolds *et al.*, 2003). Rates of Bu production observed by Sutton *et al.* (2003) were low (0.05–0.08 of total VFA produced) and probably led to low intra-epithelial Bu concentrations and a reduced rate of Bu metabolism as well. As a result, the Bu concentration gradient between the lumen and the intra-epithelial pool may have been reduced, reducing the apparent fractional rate of Bu absorption compared to that of Ac and Pr. A low Bu concentration may also have caused a lack of inhibition of Ac and Pr metabolism by Bu (Harmon *et al.*, 1991), resulting in similar fractional absorption rates of Ac and Pr. Because pH values were relatively high, concentration of uVFA will have been low and the absorption of Bu must have been caused mainly by transport of dVFA. Consequently, the lowest fractional absorption rate of Bu reported by Sutton *et al.* (2003) seems explainable only with the presumption that facilitated VFA transport is sensitive to concentration gradients as well. The results of Dijkstra *et al.* (1993) also indicated that intra-epithelial VFA metabolism affects VFA concentration gradients and needs to be involved to explain the observed differences in fractional absorption rate between individual types of VFA. At high pH value and low VFA concentrations, the fractional absorption rate of Ac was substantially lower than for Pr and Bu. This was explained by a reduced concentration gradient of Ac as a result of the relatively low intra-epithelial capacity for Ac metabolism. Furthermore, the competitive inhibition of Ac metabolism by the presence of Bu may have been involved. For Pr the effect was less prominent, corresponding to the finding that Pr metabolism is metabolised to a higher extent than Ac metabolism (discussed in next section). Results of VFA absorption rates obtained by López *et al.* (2003) in sheep generally confirm the findings of Dijkstra *et al.* (1993). In conclusion, observed effects of VFA concentration under conditions of high rumen pH

suggest that facilitated VFA transport depends on the concentration gradient of dVFA. Further, it is as assumed here that the kinetics of the mechanism of facilitated transport of individual types of VFA are identical, which leads to following change of Equation [4]:

$$U_{dVFA} = \frac{v_{\max,Ab3} Vol^{0.75}}{1 + M3/(C_{dVFA} - C_{dVFAi})} \quad [9]$$

where C_{dVFA} and C_{dVFAi} (mol/l) are the concentrations of VFA in the rumen and the intra-epithelial pool of dVFA (dVFAi), and $v_{\max,Ab3}$ and $M3$ have different values from $v_{\max,Ab2}$ and $M2$. At physiological pH, almost all intra-epithelial VFA will be in the dissociated form and hence the Equation [9] can be simplified to

$$U_{dVFA} = \frac{v_{\max,Ab3} Vol^{0.75}}{1 + M3/(C_{dVFA} - C_{VFAi})} \quad [10]$$

Transport to blood. It is assumed that transport from rumen fluid to the intra-epithelial pool is the ratelimiting step in VFA transport from lumen to blood. Subsequent transport of VFA from the intra-epithelial compartment to blood was assumed to be facilitated and much faster than the facilitated transport rate from lumen to the intra-epithelial compartment, as discussed previously by Bannink *et al.* (2000), but was represented in a similar manner to Equation [10] with $C_{dVFA} - C_{VFAi}$ replaced by the concentration difference between C_{VFAi} and that of VFA in arterial blood.

Epithelial VFA metabolism

Biology

The first step in intra-epithelial metabolism of VFA is their activation by CoA-synthetases (Rémond *et al.*, 1995). In this study it is assumed that this step is rate limiting and irreversible and determines the extent of intra-epithelial VFA metabolism. Furthermore, it is assumed that the metabolic activity of epithelial tissues is responsible for VFA metabolism by rumen tissues. Enzyme assays by Ash & Baird (1973), Harmon *et al.* (1991) and Scaife & Tichivangana (1980) demonstrate the existence of competitive inhibition among individual types of VFA in the activity of the CoA-synthetases which activate VFA to VFA-CoA as the first step of their metabolism. Based on data from Pennington (1952) similar enzymatic activities of epithelial tissue of the rumen, reticulo-rumen, omasum and abomasum is assumed (Bannink *et al.*, 2000). The data from Lobley *et al.* (1994) also indicated similar fractional rates of protein turnover in rumen and abomasal tissue. The increase in the value for the

reticulo-rumen with increased feed intake was three times larger however (from 0.22 to 0.35 /d and 0.24 to 0.28 /d in the reticulo-rumen and abomasum with sheep fed 1.25 and 2.0 times maintenance) and was probably related to expansion of the reticulo-rumen in response to a greater fill. Also, Reynolds *et al.* (2004) and Baldwin *et al.* (2004) observed selective growth of the reticulorumen specifically in dairy cows during the transition period. Although protein synthesis rate is a major determinant of the energy requirement of epithelial tissues, the facilitated transport of dVFA and ions adds significantly to this requirement (Milligan & McBride, 1985; Summers *et al.*, 1986; Gill *et al.*, 1989). Generally, with increased metabolic activity of rumen epithelia as a result of a higher VFA load that needs to be transported, the relative contribution of energy costs involved with dVFA and ion transport to total energy costs will increase (Gill *et al.*, 1989; Bannink *et al.*, 2006c). Moreover, the relative contribution of rumen tissue to total energy cost of the portal drained viscera will increase. Just as important as changes in the metabolic activity per unit mass of epithelial tissue, are changes in morphology, absorptive area (shape of rumen papillae) and epithelia mass as a response to nutrition. This aspect will be discussed later.

Modelling approaches

VFA activation rate. In general, this competitive inhibition type of reaction can be represented by the following equation:

$$P_{\text{VFA1-CoA}} = \frac{v_{\text{max,VFA1}} W}{(1 + M4/C_{\text{VFA1}}) (1 + C_{\text{VFA2}}/J)} \quad [11]$$

where $P_{\text{VFA1-CoA}}$ is rate of VFA1 activation to VFA1-CoA(mol/d), C_{VFA1} the intra-epithelial concentration (C_{VFAi}) of VFA1 (mol/l), C_{VFA2} the intra-epithelial concentration of VFA2 inhibiting activation of VFA1 (mol/l), $v_{\text{max,VFA1}}$ the maximum rate of VFA1 activation (mol / (d . g tissue mass)), W the tissue mass (g), and $M4$ and J are constants for VFA1 activation and inhibition by VFA2 (mol/l), respectively. As an illustration, Figure 2A indicates the competitive inhibition of Ac by Pr and Bu, and Figure 2B the competitive inhibition of Pr metabolism by Ac and Bu. Analysis of literature data on enzyme assays and background of parameterisations of the equations of competitive inhibition among Ac, Pr and Bu were reported earlier (Bannink *et al.*, 2000). The sensitivity of the activation rate of Ac and Pr for the concentrations of other VFA is demonstrated in Figures 2A & 2B. As discussed in the previous section on VFA absorption, Bu has a strong inhibitory effect on both Ac and Pr activation, whereas Pr, but not Ac, has an inhibitory effect as well. Activation of Bu is, however, hardly affected by Ac and Pr (Bannink *et al.*, 2000).

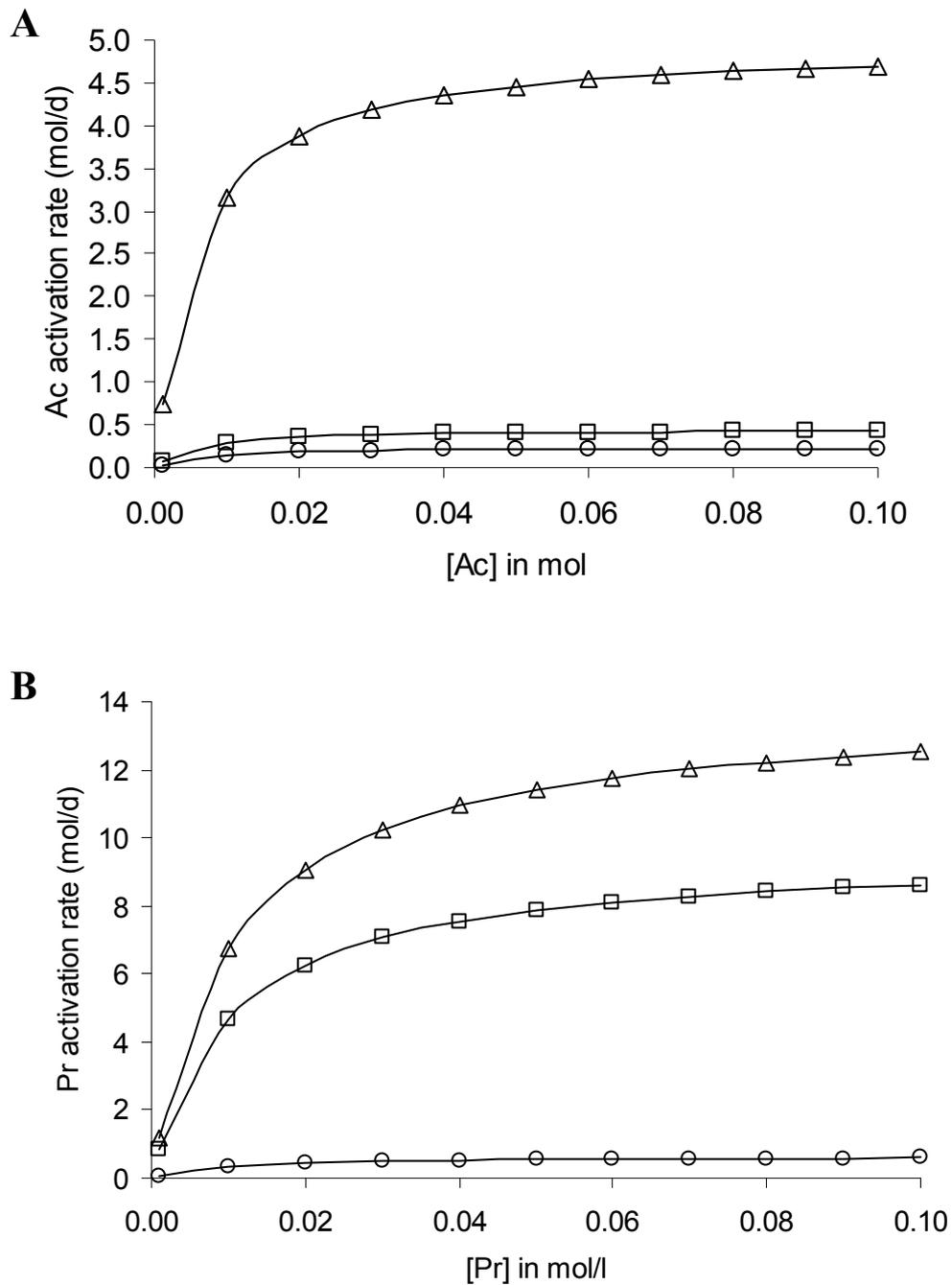


Figure 2. Activation rates of (A) acetate (mol acetyl-CoA formed/d) without other VFA (Δ), and with competitive inhibition by propionate (0.05 mol/l; \square) and butyrate (0.02 mol/l; \circ), and of (B) propionate (mol propionyl-CoA formed/d) without other VFA (Δ), and with competitive inhibition by acetate (0.09 mol/l; \square) and butyrate (0.02 mol/l; \circ), under assumption of 1500 g of epithelial tissue (Ac, Pr: acetate, propionate, respectively; adapted from Bannink *et al.*, 2000).

Epithelial metabolic activity. Assuming that all energy required by epithelia is derived from VFA metabolism, a change in total energy requirement per unit of tissue mass can be

represented by changing the value of $v_{\max, \text{VFA1}}$ in Equation [9]. The strong increase in the fractional rate of protein synthesis in rumen tissues reported by Lobley *et al.* (1994) indicates that $v_{\max, \text{VFA1}}$ may vary with feed intake level and stage of lactation, and may be in the order of 20–30% (Bannink *et al.*, 2006c). Per unit of weight of rumen tissue, VFA metabolism estimated with the parameterisation by Bannink *et al.* (2000) and shown in Figure 2 is of a similar magnitude as the oxygen consumption rates reported by Milligan & McBride (1985) corresponding to full oxidation of either 5.6, 3.1 and 2.2 mol/d of Ac, Pr and Bu, respectively. With increased metabolic activity of epithelial tissue, a proportional increase in $v_{\max, \text{VFA1}}$ for every type of VFA is assumed.

Rumen epithelium mass

Biology

The rumen epithelia need to adapt to strong increases in the load of VFA they are exposed to because of the metabolic consequences of transporting VFA and maintenance of intra-cellular homeostasis. Without a change of tissue mass, the load of VFA for an individual epithelial cell would increase dramatically. By this increase the metabolic capacity of cells to transport VFA and maintain intra-cellular homeostasis would be stretched to the maximum with the risk of damage or loss of cell or tissue integrity. Although such damage or dysfunction may occasionally occur (rumen acidosis), the response of epithelial tissues in a healthy cow is characterised by a very strong and fast adaptation to changing nutritional conditions (Dirksen *et al.*, 1984; Enemark *et al.*, 2002; Bannink *et al.*, 2005). In a recent study conducted in our own research facilities, adaptation of rumen epithelial tissue was observed in response to a strategy of a slow (treatment S) and a fast increase (treatment F) in concentrate intake by cows immediately following calving. With treatment F, the shape of rumen papillae changed faster (longer and thinner), and in first instance thickness of epithelium and keratine layer on top of the epithelium was reduced. From a teleological point of view, this seems a functional adaptation because of the increased absorptive area and a thinner epithelia barrier for VFA transport. This adaptation caused rumen VFA concentrations to be similar for both treatments despite the higher VFA production rate on treatment F during the first 2 weeks of lactation. Figure 3 shows a schematic representation of the response of the epithelia to these different rates of increase in concentrate intake, and the development in time of rumen pH and VFA concentrations. Hence, besides an effect on the metabolic activity per unit of tissue mass, the morphology of the rumen epithelia as a physical barrier to VFA absorption and epithelia mass also respond to the nutritional strategy followed. A high metabolic cost must be expected for these functional adaptations of rumen epithelia, which affects VFA absorption and VFA metabolism.

Modelling approaches

A goal-directed response of rumen epithelia (*i.e.* changes in the gene expression of enzymes, transport proteins and receptors for circulating or luminal trophic factors; Bannink *et al.*, 2006c) may be represented by a feed-back control mechanism. In this case the goal of epithelia can be represented by a so-called set-point value as a function of the VFA load to which epithelia is (or has been) exposed to. Considering the changes in epithelial morphology (Figures 3A & 3B), two types of epithelial responses need to be distinguished to represent the changes recently observed *in vivo* in lactating cows (Bannink *et al.*, 2005): (i) papillae shape or absorptive area, and (ii) epithelial tissue mass. Considering the rumen epithelia as a layer of cells on the surface of the papillae, the epithelial tissue mass can be calculated by the following equations:

$$W = Vol^{0.75} p4 s \quad [12]$$

with W is the epithelial tissue mass (g), $p4$ a factor (g/l_m) to relate W to the absorptive surface area (Vol to the exponent 0.75; see Equation [1]) and to account for adaptation of papillae size as a function of rumen VFA load of $U_{uVFA} + U_{dVFA}$ (mol/d), and s is the thickness of epithelia (μm).

The feed-back control mechanism may be represented by the following equation:

$$P_{Y_t} = (Y_g - Y_t) k1 \quad [13]$$

with P_{Y_t} is the rate of increase (“production”) of response variable Y_t , Y_g the goal or setpoint value of the response variable Y_t , Y_t the value of Y at time t , $k1$ the time constant (/d) indicating the delay in response of Y_t to reach set-point value Y_g .

The value of Y_g may be calculated by a nonlinear equation saturating at $Y_{g \max}$, and a reasonable estimate of the affinity constant $M5$ (mol/l; low and high M value for a strong and mild adaptation) of the response of Y_g to a change in VFA production rate:

$$Y_g = \frac{Y_{g \max}}{1 + (M5/U_{VFA_{\text{avg}}})^{p5}} \quad [14]$$

with $p5$ as a shape parameter and $M5$ as the affinity constant for the change of the set-point value Y_g in response to the average VFA load during the preceding period $U_{VFA_{\text{avg}}}$ (mol/d). The $U_{VFA_{\text{avg}}}$ can be calculated by the following equation with $k2$ as a parameter defining the length of the period $U_{VFA_{\text{avg}}}$ is calculated for:

$$P_{U_{VFA_{\text{avg}}}} = k2 (U_{uVFA} + U_{dVFA} - U_{VFA_{\text{avg}}}) \quad [15]$$

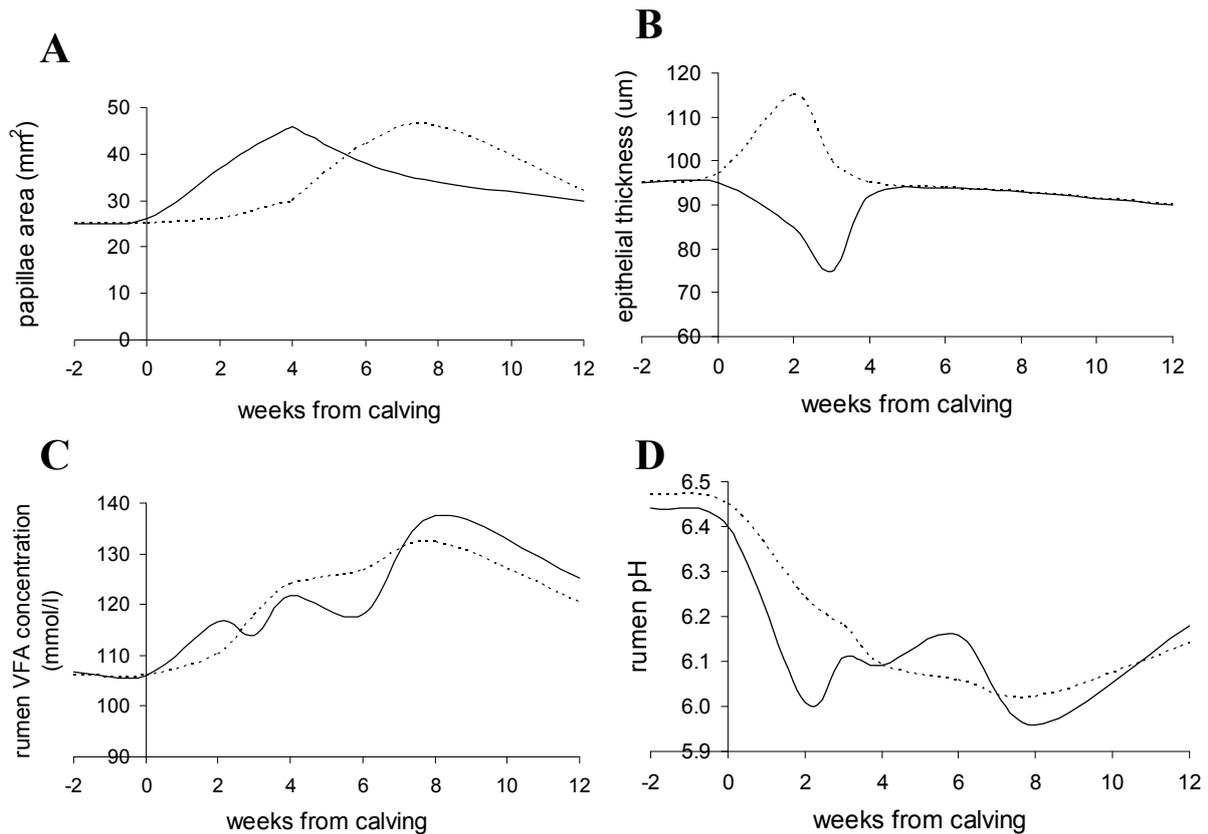


Figure 3. General trend of adaptive response of rumen epithelia with (A) papillae size and (B) thickness of epithelia, and of observed (C) rumen VFA concentrations and (D) rumen pH, to the treatment of a slow and a fast increase of concentrate intake (differing 2.0, 4.0, 2.8 and 0 kg on 6, 11, 14 and 21 days, respectively, after calving) on the same basal diet of grass silage and maize silage. Average values are shown of five cows on the slow treatment and six cows on the fast treatment (adapted from Bannink *et al.*, 2005). Solid and dashed lines indicate the observations with treatment F and S (fast and slow increase of concentrate intake; see text for further explanation).

Papillae shape. From the data illustrated in Figure 3, the rate of change of papillae shape appears to depend on the VFA load the papillae are exposed to. With treatment F, the fast increase in VFA load (maximum of 8 kg of concentrate dry matter added to basal ration at 1.5 week; 25 kg of dry matter intake per day at 3 weeks) resulted in a fast adaptation to a maximum papillae size at 3–4 weeks. With treatment S (maximum of 8 kg of concentrate dry matter intake at 3 weeks, 25 kg of dry matter intake per day at 3 weeks) maximum papillae size was observed at 6 weeks. Therefore, papillae size seems to follow the VFA load with a delay of 1–2 weeks with treatment F, and 2–3 weeks with treatment S. The faster adaptation of papillae with F may be a consequence of a faster drop in pH of rumen fluid, although rumen VFA concentrations were similar with both treatments (Figures 3C & 3D). The latter results indicate that other factors besides rumen VFA concentration influenced the rumen acid–base mechanisms (*e.g.* rumination, saliva production) and caused a less severe drop in

rumen pH with the S treatment although rumen VFA concentrations were similar. Although rumen pH might be a better candidate for modelling change in papillae size, rumen pH predictability is still poor (Pitt *et al.*, 1996; Kolver & de Veth, 2002). Therefore, rumen VFA load was chosen in the present study as the determinant factor. From the results shown in Figures 3A & 3B, it was concluded that the value of k_1 in Equation [13] should be 0.50 /d in the case of the papillae size to an increased load of VFA. Considering the fast response of epithelia to treatment F within a week, for k_2 in Equation [15] initial values of 0.75 /d were assumed to calculate the average VFA load during the preceding week. A doubling of the surface area of papillae size was estimated (Figure 3A) with $Y_{g \max} = 50 \text{ mm}^2$, $M_5 = 75 \text{ mol/d}$ and $p_5 = 5$ in Equation [14].

Epithelial mass. Applying Equation [12], from the results shown in Figures 3A & 3B it can be calculated that epithelial tissue mass W became maximal at 4 and 6 weeks with treatment F and S. This means that with a delay of 2–3 weeks the maximum response in epithelial tissue growth was achieved with both treatments. Growth of papillae size was faster than growth in epithelial mass with treatment F and slower with treatment S, resulting in an initial reduction of epithelial thickness with treatment F and an increase with treatment S (Figure 3B). Despite the fast response in change of papillae size, the minimum period of time required for changes in epithelial tissue mass seems to be about 2 weeks. From this it was concluded that the value of k_1 in Equation [13] should also be 0.25 /d in the case of the response of epithelial tissue mass to an increased load of VFA. Also a doubling of epithelial mass was assumed as epithelial thickness returned to around 100 μm (Figure 3B) within 4 weeks when papillae size was still almost doubled (Figure 3A). Therefore, in applying Equation [14] to epithelial tissue mass, $Y_{g \max}$ was taken to be equal to 3000 g, and for M_5 and p_5 the same values were assumed as with papillae size.

Feeding strategies

With a change in feeding strategy, a shift may occur in type of VFA formed (Figure 1). In Table 2 simulation results are shown for the consequences of VFA production rate and fermentation pattern on intra-epithelial VFA metabolism. In general, the contribution of individual VFA to the net appearance of VFA in portal blood is not a close reflection of the proportion of their production in the rumen (Table 2). Relatively more Ac and less Pr and Bu appears in portal blood. The proportion of rumen VFA that was metabolized during transport decreased with an increased VFA load as a result of saturation of CoA-synthetase activity. However, assumptions on the adaptation of the reticulo-rumen wall had an important effect on the extent of intra-epithelial metabolism of Ac and Pr, whereas Bu metabolism remained invariably high around 0.8 (Table 2). Increase of VFA production rate from 75 to 125 mol/d

without the assumption of epithelial adaptation caused a decrease of Pr metabolism of 6% whereas almost no decrease was simulated with adaptation. With an increased contribution of Pr to total rumen VFA production, the proportion of Pr metabolised decreased by 3%. Relative absence of Bu resulted in an increased extent of Ac and Pr metabolism as a result of less competitive inhibition by Bu (Table 2). From these simulation results it is concluded that, besides the metabolic activity of reticulo-rumen epithelia and the amounts of VFA supplied to them, the adaptive response of these epithelia is equally important for understanding the extent of VFA metabolism and portal VFA appearance, in particular of Pr as an important precursor of glucose.

Kristensen & Harmon (2004a, b) demonstrated that the intra-epithelial metabolism of Ac and Pr is much lower (less than 0.1) than the 0.3 and 0.5 for Ac and Pr previously mentioned in literature based on portal net flux measurements. Main cause of the difference is the extensive uptake by portal drained viscera of arterial Ac. Notably, also the current simulation results indicate less VFA metabolism during transport across the rumen wall (between 0.09 and 0.14 of absorbed Ac and between 0.24 and 0.36 of absorbed Pr). These outcomes are closer to the findings of Kristensen & Harmon (2004a, b) but remain higher. A possible explanation of this may be the fact that the parameterization of the CoA-synthetase activity for VFA activation was derived from the study of Harmon *et al.* (1991) conducted on rumen epithelium of calves. The reason for this choice was the completeness of the number of combinations of VFA and the different concentrations of inhibiting VFA tested (Bannink *et al.*, 2000). However, the enzyme activity for Ac and Pr activation were about twice as high as in rumen epithelium from dairy cows in the study of Ash and Baird (1973), whereas that for Bu appeared more similar. When the latter results are considered more representative for the rumen epithelium of the steers in the study of Kristensen & Harmon (2004a, b), a much lower Ac and Pr metabolism would have been predicted in the present simulation study with slightly more than 0.05 and 0.1 of Ac and Pr metabolized, respectively, which is more close to the *in vivo* findings of Kristensen & Harmon (2004a, b). This means that the apparent discrepancy of *in vitro* results and *in vivo* findings with the washed rumen model and stable isotope techniques (Kristensen *et al.*, 2000) do not become apparent to the same extent with the present modelling approach. More evidence needs to be gathered, however, on the enzymatic activity of ruminal epithelium under various physiological conditions and specific for the target animal.

Some important aspects of VFA metabolism have not been considered in the present study. Firstly, there is the possibility of interconversion between VFA through intra-epithelial metabolism as discussed by Kristensen & Harmon (2004a). Secondly, substantial amounts of arterial VFA are utilized by portal drained viscera (Kristensen & Harmon, 2004a, b). Introducing both aspects in the model would significantly change the predicted value of the contribution of absorbed VFA to net portal VFA appearance (Table 2). Further, with intra-ruminal infusions of VFA there is the possibility of microbial metabolism of VFA and

Table 2. Steady-state simulations of the effect of production rate and molar proportion of rumen VFA on extent of metabolism and molar proportion of rumen VFA appearing in portal blood.

Rate of VFA production in reticulo-rumen (mol/d)	Relative contribution individual VFA (mol/100 mol)		Extent of intra-epithelial metabolism ¹ (mol/100 mol)			Relative contribution individual VFA to net appearance of VFA in portal blood ² (mol/100 mol)		
	Ac:Pr:Bu		Ac	Pr	Bu	Ac	Pr	Bu
75 ³	70:15:15		13.5	32.9	79.3	88.8	8.6	2.7
75	50:35:15		14.2	30.3	79.1	77.8	19.4	2.8
75	60:35:5		14.2	35.7	81.3	81.5	17.4	1.1
125 ³	70:15:15		8.8	26.4	77.2	88.2	9.0	2.8
125	50:35:15		9.2	23.8	76.8	76.7	20.4	2.9
125	60:35:5		9.3	29.3	80.4	80.6	18.4	1.1
125 ⁴	70:15:15		11.9	32.3	81.0	88.9	8.6	2.5
125	50:35:15		12.5	29.1	80.8	77.9	19.6	2.6
125	60:35:5		12.6	35.1	83.0	81.5	17.5	1.0

¹ Proportion of VFA produced in the rumen that does not appear in portal blood.

² Proportion contribution of Ac, Pr and Bu to the portal-arterial concentration differences of VFA. Arterial concentrations were assumed to change proportionally with rate of VFA production (reference values of 1.400, 0.040 and 0.013 mmol/l of Ac, Pr and Bu, respectively, at a VFA production rate of 87 mol/d; Reynolds *et al.*, 1988).

³ No changes in epithelial mass and surface area were assumed compared to the situation of VFA production rates of 75 mol/d. No changes were assumed for fluid volume, rumen pH and fractional passage rate of fluid. Epithelia mass of the reticulo-rumen was 1500 g, with a surface area of papillae of 25 mm², and epithelia mass of omasum and abomasum was 1800 g.

⁴ Epithelial mass and surface area were allowed to change, resulting in an increase in reticulo-rumen epithelia mass to 2980, and an increase in the surface area of rumen papillae to 49 mm². No changes were assumed in fluid volume, rumen pH and fractional passage rate of fluid, and epithelia mass of omasum and abomasum.

Table 3. Steady-state simulations of the effect of rumen pH on VFA absorption rate and the relative contribution of facilitated VFA transport

Production rate in reticulo-rumen (mol/d)	Relative contribution individual VFA (mol/100 mol)	Rumen pH	Absorption rate from reticulo-rumen (mol/d)			Relative contribution of facilitated transport to the absorption rate of individual VFA (mol/100 mol)		
			Ac	Pr	Bu	Ac	Pr	Bu
75 ¹	60:25:15	6.3	32.3	14.3	9.0	87.0	80.2	78.8
		6.0	33.3	14.8	9.3	71.0	58.7	57.9
		5.7	34.5	15.4	9.7	59.2	44.7	45.3
125 ²	60:25:15	6.3	52.5	23.6	15.0	79.8	71.2	70.3
		6.0	54.5	24.5	15.5	68.8	57.1	57.0
		5.7	56.7	25.5	16.1	57.0	43.0	44.4
125 ³	60:25:15	6.3	55.5	24.4	15.3	81.8	72.7	71.1
		6.0	57.0	25.2	15.8	71.2	59.0	57.9
		5.7	58.7	26.0	16.3	59.4	45.1	45.3
125 ⁴	60:25:15	6.3	60.6	26.4	16.4	81.1	72.5	71.3
		6.0	61.9	27.0	16.7	70.5	58.9	58.2
		5.7	63.3	27.6	17.1	58.8	45.1	45.8

¹ Assumptions were similar to those in Table 2 for a VFA production rate of 75 mol/d.

² No change in fractional passage rate of rumen fluid, fluid volume, and of reticulo-rumen epithelia mass and surface area of papillae compared to the condition of VFA production rate of 75 mol/d.

³ Fractional passage rate of rumen fluid changed from 2.5 to 3.5 /d, fluid volume changed from 60 to 85 L.

⁴ Reticulo-rumen epithelia mass changed from 1500 to 2980 g, and surface area of papillae changed from 25 to 49 mm². No changes in fractional passage rate of rumen fluid and fluid volume.

VFA interconversions. This seems particularly important for the correct interpretation of experimental results and analysing the metabolic fate of infused VFA. The present model, however, requires net yields of individual VFA as an input, comparable to the models of rumen microbial metabolism that have been published so far.

The nutritional strategies also have an important effect on the pH of rumen fluid, which in turn affects VFA absorption rate and VFA metabolism by its effect on intra-epithelial VFA concentrations. The effect of pH on VFA absorption was studied under different sets of assumptions about VFA production rate, pH of rumen fluid, adaptation of reticulorumen epithelia, and fluid dynamics (Table 3). The results indicated that a reduction of pH from 6.3 to 5.7 had only moderate effects on the absorption rate of VFA from the reticulorumen. The relative contribution of facilitated transport of VFA reduced drastically with pH. Without changes in the rumen fluid dynamics and epithelia adaptation, similar fractions were absorbed from the reticulo-rumen. A change in the fluid dynamics (increased fluid volume and fractional outflow rate) caused only slight changes, whereas a change in epithelial mass and surface area resulted in higher proportions of VFA absorbed from the rumen. Again, it is concluded here that adaptation of the reticulo-rumen epithelia is an important mechanism for regulating the clearance of VFA from the rumen. Figure 4 illustrates the effect of epithelia adaptation during early lactation on the simulation results. As a contrast to the steady-state simulations summarised in Tables 2 & 3, the dynamics of the growth of epithelia mass and papillae size in the reticulo-rumen were simulated.

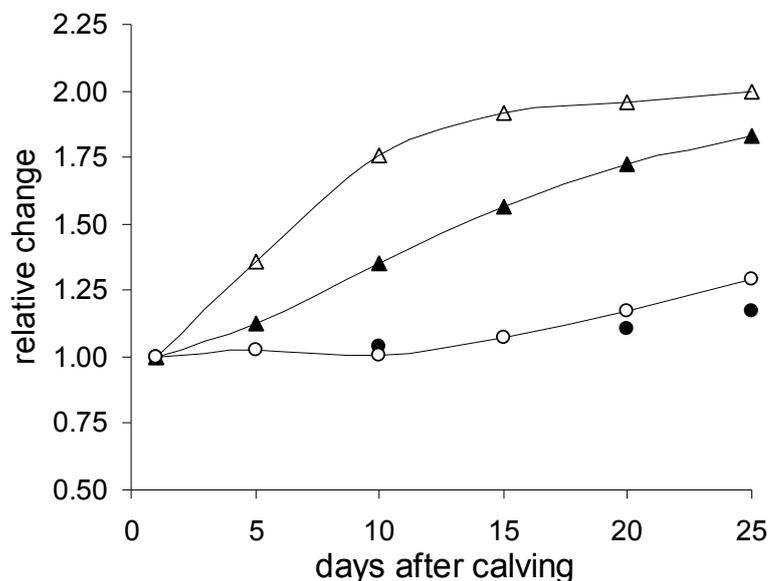


Figure 4. Non-steady-state simulation of the relative change in mass (▲) and surface area (△) of reticulo-rumen epithelia and rumen VFA concentration (○) with an estimated increase of VFA production rate from 75 to 138 mol/d during the first 25 days of lactation (observed rumen VFA concentration, ●).

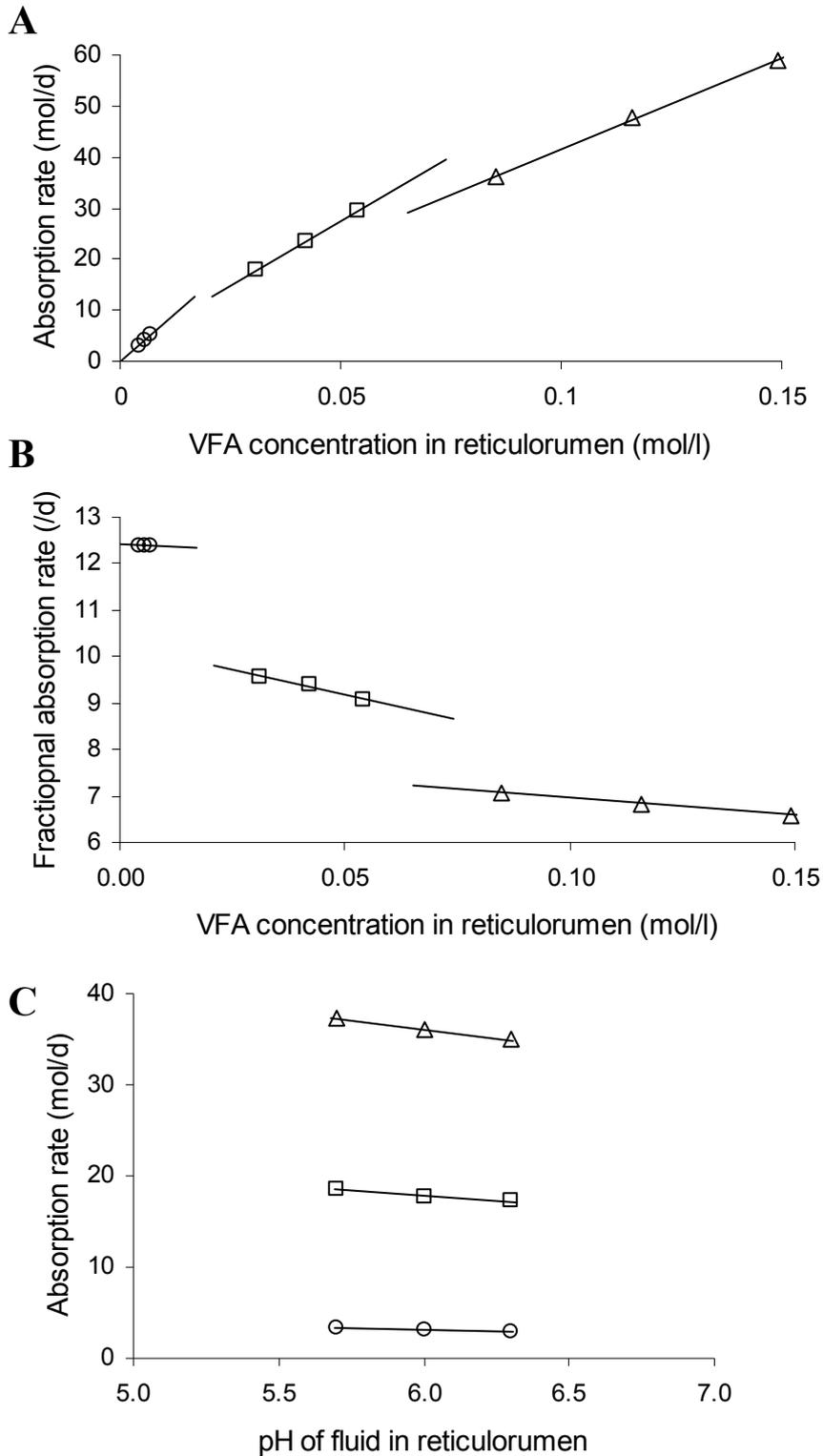


Figure 5. Steady-state simulations of the effect of VFA production rate (75, 100 and 125 mol VFA/d) on the relationship between reticulo-rumen VFA concentration and (A) VFA absorption rate, or (B) fractional rate of VFA absorption, and of the effect of pH (VFA production 75 mol/d) on (C) the relationship between pH and VFA absorption rate with 75 mol of VFA produced /d (acetate, Δ ; propionate, \square ; butyrate, \circ ; other assumptions similar to those in Table 2 for a VFA production rate of 75 mol/d).

For a linear increase of VFA production over time from 75 mol/d on the first day of lactation to 125 mol/d on the 20th day of lactation, a comparable adaptation response was simulated (Figure 4) to that observed (Figures 3A & 3B). Also, a comparable pattern of rumen VFA concentration over time was simulated, which indicates that a rapid response of the rumen papillae and epithelial mass kept VFA concentrations low despite a 67% increase in VFA production rate in a period of 20 days.

Finally, reported effects of VFA concentrations and pH on fractional VFA absorption rate in the literature can be reproduced by the modelling concepts adopted in the present study. Figure 5A shows a decrease of absorption rate in the order of Bu, Pr and Ac, which is similar to the results reported by Dijkstra *et al.* (1993), although the absorption rate of Ac was more depressed in the latter study. With an increase in rumen VFA concentration, the calculated fractional absorption rates (Figure 5B) decreased most for Pr and least for Bu. In particular, the latter result was also found and discussed by Dijkstra *et al.* (1993). Testing the effect of decreased pH resulted in reductions in absorption rate of Ac, Pr and Bu that were comparable to those established by Dijkstra *et al.* (1993) for this range of pH (Figure 5C). With a reduction of pH, the rumen concentration of Ac decreased from 0.092 to 0.076 mol/l, those of Pr from 0.035 to 0.027 mol/l, and those of Bu from 0.005 to 0.004 mol/l. These changes were accompanied by a 23, 29 and 32% increase in the fractional absorption rate of Ac, Pr and Bu, respectively, comparable to that reported by Dijkstra *et al.* (1993).

Conclusions

Inclusion of a representation of the functionality of the rumen wall in models of rumen function is likely to improve the range in which models of rumen function can be applied. It is likely that interactions between VFA formation, VFA absorption, intra-epithelial VFA metabolism and adaptation of epithelia are important determinants of observed effects of feeding strategy on rumen functioning and nutrient availability. The model described seems useful for testing hypotheses on rumen function, but needs extension and further testing against experimental observations. There is particular need, however, for more solid data for appropriate parameterisation of the model. Model elements which require specific attention in future experimental research are the activity of the CoA-synthetases, the VFA specificity of these enzymes, the existence of competitive inhibition among individual VFA, and the preferential use of nutrients for biosynthetic and maintenance functions under various nutritional conditions and for various physiological conditions and ruminant types. Also, further information is needed on the relationship between the adaptive response of the rumen wall and the implications for its absorptive capacity, its capacity of VFA metabolism and its energy requirements. Modelling the interaction between the functions of the reticulo-rumen wall and the intra-luminal fermentative processes requires the present model to be combined with existing models of rumen function.

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References

- Argyle, J.L. & Baldwin, R.L., 1988. Modeling of rumen water kinetics and effects of rumen pH changes. *Journal of Dairy Science* 71, 1178-1188.
- Ash, R. & Baird, G.D., 1973. Activation of volatile fatty acids in bovine liver and rumen epithelium. *Biochemical Journal* 136, 311-319.
- Baláz, Š., 2000. Lipophylicity in trans-bilayer transport and subcellular pharmacokinetics. *Perspectives Drug Discovery Research* 19, 157-177.
- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating cow II. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54, 107-131.
- Baldwin, R.L., 1995. *Modeling Ruminant Digestion and Metabolism*. Chapman & Hall Ltd., London, United Kingdom, pp. 578.
- Baldwin, R.L., McLeod, K.R. & Capuco, A.V., 2004. Visceral tissue growth and proliferation during the bovine lactation cycle. *Journal of Dairy Science* 87, 2977-2986.
- Bannink, A. & De Visser, H., 1997. Comparison of mechanistic rumen models on mathematical formulation of extramicrobial and microbial processes. *Journal of Dairy Science* 80, 1296-1314.
- Bannink, A., De Visser, H., Klop, A., Dijkstra, J., France, J., 1997. Causes of inaccurate prediction of volatile fatty acids by simulation models of rumen function in lactating cows. *Journal of Theoretical Biology* 189, 353-366.
- Bannink, A., Kogut, J., Dijkstra, J., France, J., Tamminga, S. & Van Vuuren, A.M., 2000. Modelling production and portal appearance of volatile fatty acids in cows. In: McNamara, J.P., France, J., Beever, D.E. (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 87-102.
- Bannink, A. & Tamminga, S., 2005. Rumen function. In: Dijkstra, J., Forbes, J., France, J. (Eds.), *Quantitative Aspects of Ruminant Digestion and Metabolism*, second edition, CAB International, Wallingford, United Kingdom, pp. 263-288.
- Bannink, A., Van Leeuwen, P., Hamminga, A., Gerrits, W.J.J., Valk, H., Stockhofe-Zurwieden, N. & Van Vuuren, A.M., 2005. The effect of feeding strategy after parturition on the development of rumen epithelium in dairy cows. In: Proceedings of the 9th International Congress of the European Society of Veterinary & Comparative Nutrition, 22–24 September, Grugliasco, Turin, Italy.

- Bannink, A., Kogut, J., Dijkstra, J., Kebreab, E., France, J., Tamminga, A. & Van Vuuren, A.M., 2006a. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. *Journal of Theoretical Biology* 238, 36-51.
- Bannink, A., Dijkstra, J., Kebreab, E. & France, J., 2006b. Advantages of a dynamical approach to rumen function to help resolve environmental issues. In: E. Kebreab, J., Dijkstra, J., France, A., Bannink & W.J.J. Gerrits (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 281-298.
- Bannink, A., Dijkstra, J., Koopmans, S.-J. & Mroz, Z., 2006c. Physiology, regulation and multifunctional activity of the gut wall, a rationale for multicompartmental modelling. *Nutrition Research Reviews* 19, 227-253.
- Chilibroste, P., Dijkstra, J. & Tamminga, S., 2001. Design and evaluation of a non-steady state rumen model. *Netherlands Journal of Agricultural Science* 49, 297-312.
- Dijkstra, J., Neal, H.D.StC., Beever, D.E. & France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *Journal of Nutrition* 122, 2239-2256.
- Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M. & Tamminga, S., 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *British Journal of Nutrition* 69, 385-396.
- Dijkstra, J., Mills, J.A.N. & France, J., 2002. The role of dynamic modelling in understanding the microbial contribution to rumen function. *Nutrition Research Reviews* 15, 67-90.
- Dirksen, G., Liebich, H.G., Brosi, G., Hagemeister, H. & Mayer, E., 1984. Morphologie der pansenschleimhaut und fettsaureresorption beim Rind- bedeutende faktoren fur gesundheit und Leistung. *Zentralblatt fur Veterinarmedizin A* 31, 414-430.
- Enemark, J.M., Jorgensen, R.J. & Enemark, P.St., 2002. Rumen acidosis with special emphasis on diagnostic aspects of subclinical rumen acidosis: a review. *Veterinarija ir Zootechnika* 20, 16-29.
- Friggens, N.C., Oldham, J.D., Dewhurst, R.J. & Horgan, G., 1998. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage. *Journal of Dairy Science* 81, 3350-3369.
- Gill, M., France, J., Summers, M., McBride, B.W. & Milligan, L.P., 1989. Simulation of the energy costs associated with protein turnover and Na⁺, K⁺ transport in growing lambs. *Journal of Nutrition* 119, 1287-1299.
- Hanigan, M.D., 2005. Quantitative aspects of ruminant splanchnic metabolism as related to predicting animal performance. *Animal Science* 80, 23-32.
- Harmon, D.L., Gross, K.L., Krehbiel, C.R., Kreikemeier, K.K., Bauer, M.L. & Britton, R.A., 1991. Influence of dietary forage and energy intake on metabolism and ayl-CoA synthetase activity in bovine ruminal epithelial tissue. *Journal of Animal Science* 69, 4117-4127.

- Kebreab, E., Mills, J.A.N., Crompton, L.A., Bannink, A., Dijkstra, J., Gerrits, W.J.J. & France, J., 2004. An integrated mathematical model to evaluate nutrient partition in dairy cattle between the animal and its environment. *Animal Feed Science and Technology* 112, 131-154.
- Kohn, R.A. & Boston, R.C., 2000. The role of thermodynamics in controlling rumen metabolism. In: McNamara, J.P., France, J., Beever, D.E. (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 11-24.
- Kolver, E.S. & de Veth, M.J., 2002. Prediction of ruminal pH from pasture-based diets. *Journal of Dairy Science* 85, 1255-1266.
- Kristensen, N.B., Pierzynowski, S.G. & Danfær, A., 2000. Net portal appearance of volatile fatty acids in sheep intraruminally infused with mixtures of acetate, propionate, butyrate, and valerate. *Journal of Animal Science* 78, 1372-1379.
- Kristensen, N.B. & Harmon, D.L., 2004a. Splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. *Journal of Animal Science* 82, 2033-2042.
- Kristensen, N.B. & Harmon, D.L., 2004b. Effect of ruminal butyrate absorption on splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. *Journal of Animal Science* 82, 3549-3559.
- Lescoat, P. & Sauvant, D., 1995. Development of a mechanistic model for rumen digestion validated using duodenal flux of amino acids. *Reproduction Nutrition Development* 35, 45-70.
- Lindsay, D.B. & Reynolds, C.K., 2005. Metabolism of the portal-drained viscera and liver. In: Dijkstra, J., Forbes, J.M., France, J. (Eds.), *Quantitative Aspects of Ruminant Digestion and Metabolism*, second edition, CAB International, Wallingford, United Kingdom, pp. 311-343.
- Lobley, G.E., Connell, A., Milne, E., Newman, A.M., Ewing, T.A., 1994. Protein synthesis in splanchnic tissues of sheep offered two levels of intake. *British Journal of Nutrition* 71, 3-12.
- López, S., Hovell, F.D.D., Dijkstra, J. & France, J., 2003. Effects of volatile fatty acid supply on their absorption and on water kinetics in the rumen of sheep sustained by intragastric infusions. *Journal of Animal Science* 81, 2609-2616.
- Milligan, L.P. & McBride, B.W., 1985. Energy costs of ion pumping by animal tissues. *Journal of Nutrition* 115, 1374-1382.
- Mills, J.A.N., Dijkstra, J., Bannink, A., Cammell, S.B., Kebreab, E. & France, J., 2001. A mechanistic model of whole tract digestion and methanogenesis in the lactating dairy cow: model development, evaluation and application. *Journal of Animal Science* 81, 3141-3150.

- Murphy, M.R., Baldwin, R.L., Koong, L.J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *Journal of Animal Science* 55, 279-296.
- Neal, H.D.StC., Dijkstra, J. & Gill, M., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model evaluation. *Journal of Nutrition* 122, 2257-2272.
- Offner, A. & Sauvant, D., 2004. Comparative evaluation of the Molly CNCPS and LES rumen models. *Animal Feed Science and Technology* 112, 107-130.
- Pennington, R.J., 1952. The metabolism of short-chain fatty acids in the sheep 1. Fatty acid utilization and ketone body production by rumen epithelium and other tissues. *Biochemical Journal* 51, 251-258.
- Pitt, R.E., Van Kessel, J.S., Fox, D.G., Pell, A.N., Barry, M.C. & Van Soest, P.J., 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. *Journal of Animal Science* 74, 226-244.
- Rémond, D., Ortigues, I. & Jouany, J.-P., 1995. Energy substrates for the rumen epithelium. *Proceedings Nutrition Society* 54, 95-105.
- Reynolds, C.K., Huntington, G.B., Tyrrell, H.F. & Reynolds, P.J., 1988. Net metabolism of volatile fatty acids, d- β -hydroxybutyrate, nonesterified fatty acids, and blood gasses by portal-drained viscera and liver of lactating Holstein cows. *Journal of Dairy Science* 71, 2395-2405.
- Reynolds, C.K., Aikman, P.C., Lupoli, B., Humphries, D.J. & Beever, D.E., 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *Journal of Dairy Science* 86, 1201-1217.
- Reynolds, C.K., Dürst, B., Lupoli, B., Humphries, D.J. & Beever, D.E., 2004. Visceral tissue mass and rumen volume in dairy cows during the transition from late gestation to early lactation. *Journal of Dairy Science* 87, 961-971.
- Scaife, J.R. & Tichivangana, J.Z., 1980. Short chain acyl-CoA synthetases in ovine rumen epithelium. *Biochimica et Biophysica Acta* 619, 445-450.
- Suàrez, B.J., Van Reenen, C.G., Gerrits, W.J.J., Stockhove, N., Van Vuuren, A.M. & Dijkstra, J., 2006. Effects of supplementing concentrates differing in carbohydrate composition to veal calf diets: II. Rumen development. *Journal of Dairy Science* 89, 4376-4386.
- Summers, M., McBride, B.W. & Milligan, L.P., 1986. Components of basal energy expenditure. In: A. Dobson & M.J. Dobson, M.J. (Eds.), *Aspects of Digestive Physiology in Ruminants*. Comstock Publishing Associates, Ithaca, United States of America, pp. 257-285.
- Sutton, J.D., Dhanoa, M.S., Morant, S.V., France, J., Napper, D.J., Schuller, E., 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *Journal of Dairy Science* 86, 3620-3633.

Sveinbjörnsson, J., Huhtanen, P., Udén, P., 2006. The Nordic Dairy CowModel, Karoline - development of volatile fatty acids sub-model. In: E. Kebreab, J. Dijkstra, A. Bannink, W.J.J. Gerrits & J. France, J. (Eds.), *Nutrient Digestion and Utilization in Farm Animals. Modelling Approaches*. CAB International, Wallingford, United Kingdom, pp. 1-14.

Chapter 8

General Discussion

General Discussion

Effects of feeding strategies on cow performance can be largely attributed to rumen function. Rumen function is not only relevant to feed digestion and lactational performance of cows, it also indirectly affects most aspects of management on dairy farms (production of forage crops, manure storage and application, feed and fertilizer import, environmental impact). For these reasons, a previous thesis was directed at mathematical modelling and integration of rumen fermentation processes using a mechanistic, dynamic approach (Dijkstra, 1993).

A mechanistic, dynamic approach in modelling rumen processes adds value because of the explanatory and integrative capacity it offers. Mechanisms that accommodate the variation in intra-ruminal processes and physiological responses to various nutritional strategies can be represented. The major factors that determine such variation have to be identified in the model in order to explain rumen function. Dynamic models that describe the underlying mechanisms have large potential for the development of strategic instruments to understand (Dijkstra *et al.*, 2002), tactical instruments to predict (Bannink *et al.*, 2006a) and operational instruments to control (Kristensen, 2005) or survey (Dijkstra *et al.*, 2007b).

The aim of the present study was to evaluate and improve previous models of rumen function. The first part of this discussion addresses the importance of rumen function, the need to model rumen function and the performance of dynamic rumen models. Subsequently, the importance of volatile fatty acids (VFA) will be discussed. This aspect is generally considered a major weakness of current models but is of large importance as VFA account for the majority of metabolizable energy available to the cow. In the final part, implications of the present study for the development of nutrient based feed evaluation systems and possibilities for anticipating the consequences of nutritional strategies will be discussed.

Anticipating instead of reacting

Lactational performance of cows may closely and rapidly (within a day) follow variation in feed intake. Despite this close association, the cow changes during the course of her lactation depending on feeding strategy and physiological characteristics. Although some control of lactational performance is possible, lactation is not a reversible process that can be controlled freely to every desired state. Hence, there are good reasons to anticipate and to attempt to predict performance beforehand, instead of reacting afterwards. Several aspects of whole dairy farm management should be evaluated in a much earlier phase than during the course of lactation, ranging from application of manure and artificial fertilizers, forage harvesting strategy, to choice between alternative forage crops and imports of concentrates

and by-products. Predictions are needed of the production response to variation in the quality of home-grown forages, of the amounts and characteristics of concentrates and by-products to be purchased, of the quantity and quality of stored cattle manure obtained, and eventually, of the surplus of nitrogen and phosphorus per hectare and related emissions to the environment. Urea content of milk may be added to this list because of Dutch legislation on manure application and ammonia emissions.

Prediction of cow performance as well as quantity and composition of cow excreta requires knowledge of the physiological mechanisms that underlie the effects of feeding strategy on digestion, nutrients available for absorption and milk synthesis. Digestibility of feeds is the basis of all feed evaluation systems currently applied in practice. Apart from extreme production conditions (e.g. disease, under-nutrition), it is generally accepted among ruminant nutritionists that the rumen plays a very important role with respect to extent and site of digestion, to the profile of nutrients absorbed from the gastrointestinal tract and to cow performance.

Dynamic modelling of rumen function

Several models have been developed that aim to predict rumen fermentation processes. The main emphasis in these models is the quantification of microbial degradation of feed substrates, microbial protein synthesis and sometimes VFA production. As already explained in Chapter 1, although all models use kinetic parameters such as fractional rates of substrate degradation as input, or preset parameter values, only few of them can be considered truly dynamic. Numerous dynamic models have been published but for many of them the mathematical representation can easily be reduced to a static one without any loss of information.

At the start of the present study, three truly mechanistic, dynamic models were available from literature: the model of Danfær (1990), the model of Dijkstra *et al.* (1992) and its subsequent version incorporating protozoal dynamics (Dijkstra, 1994) and the model of Baldwin *et al.* (1987) and its subsequent versions (Baldwin, 1995), the latter two being successors of earlier exercises by France *et al.* (1982) and Baldwin *et al.* (1970). A detailed comparison of dynamic models was not available from previous studies (Ramangasoavina & Sauvant, 1993; Kohn *et al.*, 1994). Comparing the impact of problematic or unknown parameter inputs (Chapter 2), mathematical representation of substrate degradation and growth of amylolytic and fibrolytic micro-organisms (Chapter 3) and prediction accuracy (Chapter 4) revealed that the models of Baldwin *et al.* (1987) and Dijkstra *et al.* (1992) were more robust and that their model outputs were more consistent with current insights into rumen function given in the literature. The Dijkstra *et al.* (1992) model contained the most essential elements, however, and was most pragmatic as well, in the sense that all of its inputs

are based on parameters actually measured in rumen trials. The Baldwin *et al.* (1987) model lacks input of substrate degradation characteristics and aims to give a more elaborate representation of particle comminution and the interaction between particles and micro-organisms. The representation of these aspects is justified because they may affect both substrate degradability and substrate passage (Bannink & Tamminga, 2005). However, unless passage rate is coupled to a mechanism for particle comminution (e.g. coupling to the reciprocal of the fractions of small and large particles, as discussed in Chapters 2 & 5), it does not give any further explanation of variation in rate of passage of particles.

Advantages of a dynamic approach

The advantages of a mechanistic, dynamic approach can be demonstrated by comparing predicted digestion of Neutral Detergent Fibre (NDF) in the rumen by the Dijkstra *et al.* (1992) model with direct calculations from *in situ* degradation characteristics and observed fractional passage rate that were used as an input to the model (see further explanation in Figure 1). The comparison indicates a more accurate prediction of rumen NDF digestion with the model of Dijkstra *et al.* (1992), which was on average 8.7% higher than observed (SD 4.5%; root of mean squared prediction error, rMSPE, of 9.6%), whereas the direct calculations were 17.7% lower (SD 5.8%; rMSPE of 18.5%). Direct calculations with more realistic estimates of NDF passage rate obtained by halving the values of fractional passage rates based on Cr-mordanted NDF, resulting from a recent comparison by Pellikaan (2004) of ¹³C-labeled NDF as an intrinsic marker with Cr-mordanted NDF as an external marker, resulted in 5.4% lower predictions than observed with increased variation (SD 6.1%; rMSPE of 7.8%). These results suggest, first, that besides *in situ* degradation characteristics and fractional passage rates, the representation of rumen fermentation conditions is also relevant (including concentrations of substrates, different types of micro-organisms and metabolites, and pH). Better reproduction of the trend in NDF digestion by the dynamic model has to be attributed to dynamic representation of the effects of rumen fermentation conditions on microbial activity and NDF degradation. Second, direct calculations appear useful only if realistic estimates of the fractional passage rate are used, in correspondence with the concepts recently adopted in the renewed DVE/OEB system for intestinally degradable protein (Tamminga *et al.*, 2007a).

A further indication of the importance of rumen fermentation conditions is the finding that results of *in situ* incubations and *in vivo* fractional rate of NDF degradation match more closely when the incubations are performed under the actual *in vivo* rumen conditions. This is illustrated in Figure 2 with the results of Valk *et al.* (1996). *In situ* incubations were performed in the same cows on the same diet in which the *in vivo* measurements of fractional rate of NDF degradation were obtained. Both results match closely. Results of the studies by Van Vuuren *et al.* (1992, 1993) do not indicate such a match, probably because the *in situ*

incubations were performed under different rumen fermentation conditions and in different animals.

To conclude, both examples illustrate the importance of fermentation conditions in the rumen to microbial activity and substrate degradation. Hence, factors characterising these conditions have to be represented in a rumen model for it to predict accurately. A dynamic modelling approach qualifies best for this purpose, in particular when relationships are essentially nonlinear. The next section will discuss important elements required in rumen modelling and the current state of the art.

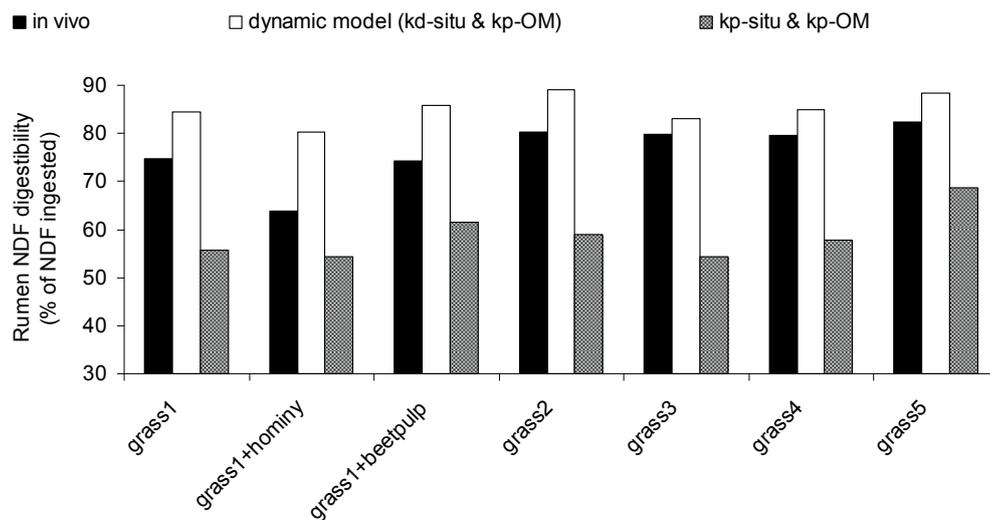


Figure 1. Comparison between observed (*in vivo*) and calculated rumen NDF digestion by the dynamic, mechanistic model of Dijkstra *et al.*, 1992 (dynamic model; *in situ* fractional rate of NDF degradation and observed fractional outflow rate of organic matter (OM) as inputs), and calculation with the ratio of *in situ* fractional rate of NDF degradation and the sum of *in situ* fractional rate of NDF degradation and *in vivo* fractional rate of OM outflow. Grass1, grass+ hominy and grass+beet pulp refer to the dietary treatments tested by Van Vuuren *et al.* (1993), whereas grass2 up to grass5 refer to the treatments tested by Van Vuuren *et al.* (1992). The data used correspond to those used for the studies described in Chapters 1, 2 & 3.

Essential elements of a rumen model

Almost every rumen model addresses the key processes in rumen fermentation, viz. degradation and outflow of various classes of substrates, growth and outflow of various functional classes of micro-organisms, and quantity and profile of VFA formed. However, models clearly differ in extent to which these processes are predicted as model outcomes or are predefined and treated as model inputs.

Microbial growth

Non-dynamic models of microbial growth generally adopt a constant efficiency of microbial growth and must be considered empirical rather than mechanistic. Even the model

of Russell *et al.* (1992), with many mechanistic elements, applies a constant efficiency of microbial growth dependent on the fractional degradation rate of substrate among other things. Dijkstra *et al.* (1998) reviewed various approaches to representing microbial growth efficiency and argued on the basis of theoretical principles that it is related to fractional passage rate rather than fractional degradation rate. Both the dynamic models of Baldwin *et al.* (1987) and Dijkstra *et al.* (1992) apply this concept and represent a mechanism for variable growth efficiency by distinction of substrate utilization for growth and non-growth purposes (Chapter 3). Also the model of Danfær (1990) adopts a concept of variable growth efficiency, but its representation does not comply with those used in literature on microbial cultures (direct coupling of nutrient requirement and microbial composition). More recently, Danfær *et al.* (2006) abandoned the concept and used a similar approach to other (non-dynamic) rumen models.

Only the model of Dijkstra *et al.* (1992) represents intra-ruminal recycling of microbial matter (Chapter 2). It is well established that defaunating the rumen may increase the efficiency of microbial growth (Jouany *et al.*, 1989; Firkins *et al.*, 2007). By representing the process of predation of bacteria by protozoa and the effect of dietary composition on the presence of protozoa, the model is capable of reproducing the effects of intra-ruminal recycling on substrate degradation and apparent efficiency of microbial growth (Dijkstra, 1994).

Finally, only the model of Dijkstra *et al.* (1992) includes a mechanism for variable microbial composition by representing the storage of polysaccharides (microbial starch). Upon reduction of the starch content of a diet, the contribution of microbial starch to duodenal starch flow increases (Bannink & Tamminga, 2005). This representation is also important with respect to simulation of feed intake patterns where the storage of microbial starch may be an important buffering mechanism that prevents immediate fermentation of these carbohydrates after the consumption of a meal. The model of Danfær (1990) also allows variation in microbial composition, but the variation in predicted starch content remained minor. Model evaluations with the data of Van Vuuren *et al.* (1992, 1993) showed that such a low microbial starch content appeared realistic, whereas the Dijkstra *et al.* (1992) and Baldwin *et al.* (1987) models over-predicted duodenal starch flow (Chapter 4). But, clearly unrealistic is the increase in predicted microbial starch flow by the model of Danfær (1990) with decreasing dietary content of rapidly fermentable carbohydrates (Chapter 2).

Types of micro-organisms and substrate specificity

All rumen models distinguish between microbial growth on rapidly fermentable carbohydrates and on structural carbohydrates. Different parameterization has to be applied for maximum growth yields and maintenance requirements. In addition, more mechanistic models distinguish between ammonia and protein as sources of N for microbial protein synthesis. The model of Danfær (1990) takes a distinct position again in that no fixed

stoichiometry for synthesis of microbial matter is used and no distinction is made between microbial growth from various sources of carbohydrate and from protein. When all 'extra-microbial' differences in the model representations were excluded (Chapter 3), the models of Baldwin *et al.* (1987) and Dijkstra *et al.* (1992) predicted very similar rates of outflow of microbial matter. However, the model of Danfær (1990) strongly deviated from them and predicted a different partition between microbial protein synthesis on ammonia and protein as sources of N.

Feed-specific intrinsic degradation characteristics of substrates

Only the Dijkstra *et al.* (1992) model requires input data from *in situ* incubations trials. These inputs are feed-specific intrinsic degradation characteristics and hence the model requires them to be measured under standardized conditions in the rumen (in contrast to the data of Valk *et al.*, 1996, but in correspondence with those of Van Vuuren *et al.*, 1992, 1993; Figure 2). The use of these inputs is a pragmatic solution to the problem of how to represent the complex mechanisms of attachment to and colonization of substrate particles by micro-organisms, and the influence of particle size and characteristics. Thus far, these processes appear too complex to be represented separately in a model of whole rumen function. Furthermore, it is not known how to derive information on these mechanisms from *in vivo* observations. *In situ* results are thought to give a reasonable indication of the final outcome to this complexity and may serve the purpose. The results shown in Figure 2 indicate this expectation is realistic to at least some extent. It remains difficult to prove, however, and experiments should not aim to validate *in situ* (and *in vitro*) by direct comparison with *in vivo* measurements. The latter is just not possible.

In contrast to the approach in the model of Dijkstra *et al.* (1992), which uses feed-specific degradation characteristics of different substrates, the model of Baldwin *et al.* (1987) assumes constant intrinsic degradation characteristics of substrates and variation in degradation rate depends fully on changes in the distribution of micro-organisms over fluid, small particles and large particles and substrate availability (Chapter 2). Without *in situ* degradation characteristics as an input, the model in itself has to explain any variation in substrate degradation (Baldwin, 1995). It is doubtful, however, whether the model is able to reproduce the variation encountered in practice with intrinsic degradation characteristics alone. Probably recognizing this limitation, Hanigan *et al.* (2006) introduced *in situ* degradation characteristics as an input to the model. This addition, however, introduces the risk that the attempt to explain variation in degradation rate characteristics is made twice. This problem does not apply to the model of Dijkstra *et al.* (1992; Chapters 2 & 3).

The original model of Danfær (1990) assumes constant degradation characteristics for carbohydrates without any consideration of their origin. Furthermore, the representation appeared not to follow generally accepted physiological responses observed *in vivo*. Recently, Danfær *et al.* (2006) developed another rumen model and, like Hanigan *et al.* (2006),

introduced *in situ* degradation characteristics as an input to the model, following the approach of almost all other models. The dynamic approach was abandoned however.

Passage characteristics

All rumen models account for the effect of passage rate on substrate degradation. Together with the intrinsic degradation characteristics, fractional passage rate is the most important determinant of substrate availability for microbial use. Fractional passage rate varies with dietary composition and feed intake level (Seo *et al.*, 2006), and probably with meal sizes, feed intake patterns, and ruminant species involved. Despite its importance, rumen models consider fractional passage rate of fluid and particulate matter as a constant model input. This is a major simplification, however, of the mechanisms underlying passage and microbial activity in the rumen *in vivo*. It has been suggested that fractional rates of passage may be related to the fractional degradation rate as an intrinsic substrate characteristic. Results of Pellikaan (2004) with intrinsic substrate markers suggest this might indeed be the case. The effect of degradation (rate) on the functional specific weight of substrate particles, caused by varying amounts of adhering fermentation gases, was proposed as an explanation for this relationship. Attention needs to be given, however, to the mechanisms responsible for passage of substrate and micro-organisms, as well as those involved with microbial colonization of particles and the different niches to be colonized in plant material (Kingston-Smith *et al.*, 2003).

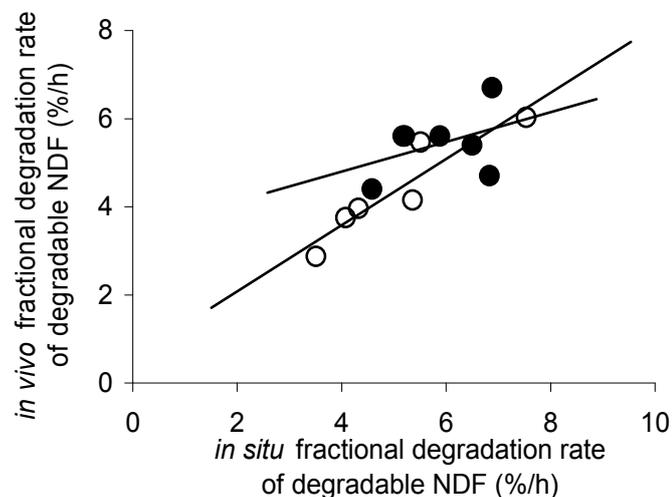


Figure 2. Comparison of fractional degradation rate of rumen NDF pool (%/h) estimated from *in vivo* observations and results of *in situ* incubation. Valk *et al.* (1996; open symbols; *in vivo* degradation rate = $0.75 \times$ *in situ* degradation rate + 0.57, $R^2=0.86$) estimated *in situ* and *in vivo* degradation for six grass treatments by rumen evacuation studies (unpublished) and *in situ* incubations in the same cows on the same dietary material. *In vivo* fractional degradation rate was estimated by subtracting fractional clearance of rumen Indigestible Acid Detergent Fibre as a marker for NDF outflow from fractional clearance of rumen NDF. Van Vuuren *et al.* (1992, 1993; closed symbols; *in vivo* degradation rate = $0.34 \times$ *in situ* degradation rate + 3.45, $R^2=0.17$) used different cows and dietary material to estimate *in vivo* and *in situ* degradation for the seven grass-based treatments which were also used for model evaluation in the present study (Chapters 1, 2 & 3). *In vivo* fractional degradation rate was estimated by subtracting fractional duodenal flow of rumen NDF from the fractional clearance rate of rumen NDF.

The mechanism for reduction of particle size in the model of Baldwin *et al.* (1987) determines the distribution of micro-organisms over fluid, small and large particles, and thereby the concentration of micro-organisms degrading small particles. However, with highly digestible diets and realistic estimates of the fraction of small particles, only slight effects of changes in particle size and comminution rate on rumen outflow were simulated (Chapters 2 & 3). The mechanism appears particularly important for a correct prediction of the size of rumen pools in response to level and pattern of feed intake (Chapter 4). When, in contrast to the approach of Baldwin *et al.* (1987), the representation of substrate degradability is unrelated to the mechanism of particle dynamics, predicted effects of particle dynamics on substrate degradation become minor (Chapter 5, Colloa-Saenz *et al.*, 2006; Bannink & Dijkstra, 2007).

Additional influencing factors

Most rumen models take into account the effect of substrate degradation characteristics, passage rate and microbial growth characteristics. However, more factors are relevant to explain rumen function. Firstly, all non-dynamic rumen models (Russell *et al.*, 1992; the mechanistic option of Pitt *et al.*, 1996; Lescoat *et al.*, 1995; Van Straalen *et al.*, 1995; the original version of the dynamic model of Danfær, 1990) adopt equal fractional rates of substrate degradation per unit of feed consumed at all levels of feed intake. However, the level of feed intake does affect rumen fermentation conditions, such as the concentration of substrate and fermenting micro-organisms present in the rumen. In this respect, dry matter intake is an important determinant factor next to the other driving variables and parameter inputs. Its effect is included only in the rumen models of Baldwin *et al.* (1987) and Dijkstra *et al.* (1992), or their predecessors, however.

Secondly, most modelling studies are limited to the simulation of steady-state conditions. A few exceptions have been reported, with varying levels of detail represented in the model (France *et al.*, 1982; Pitt *et al.*, 1996; Chapter 5). A recent modelling study (Bannink & Dijkstra, 2007) indicated that for practical situations (e.g. frequent and mixed feeding), the simulation of ruminal non-steady states is much less important than proper representation of the processes of substrate degradation and microbial growth. Although this may hold in general, there are some consequences of feed intake pattern worthy of mention. Simulations indicate that with frequent feeding the synchronisation of energy and N supply to micro-organisms has a limited effect on rumen function as long as rumen fermentation conditions, such as pH, passage rates and fluid, volume are kept constant (Bannink & Dijkstra, 2007). Previous modelling efforts by Bannink & Tamminga (2005), with different concepts adopted in the model, led to the same conclusion. This suggests that the effects of synchronizing diets should perhaps be sought in the area of changes in rumen fermentation conditions (e.g. to avoid low rumen pH), or perhaps that of feedback regulation of feed intake. The simulated outcomes clearly do not support the concept of beneficial effects of

synchronization as suggested in the literature, and appear in line with the effects established *in vivo* but not *in vitro* (Casper *et al.*, 1999; Rotger *et al.*, 2006). Conversely, simulations with only a few starch-rich meals per day indicated a strong impact of the number of meals on ruminal starch digestion, whereas that of NDF and protein remained hardly affected. This result suggests that highly discontinuous availability of starch substrate (one or two starch meals during the day, or a highly asynchronous diet) may lead to strong fluctuation in the population size of starch degrading micro-organisms, leading to a low starch digestion capacity at the moment large amounts of starch become available in the rumen. The simulation results appear to be confirmed by reported *in vivo* data on the effect of number of meals on rumen starch digestion (Bannink & Dijkstra, 2007; Figure 3). Such effects need to be explored further, preferably in combination with passage out of the rumen and the effects of meal size and pattern.

Thirdly, in many modelling attempts the effect of pH of rumen fluid on cell wall degradation has been introduced (Argyle & Baldwin, 1988; Dijkstra *et al.*, 1992; Pitt *et al.*, 1996; Lescoat & Sauvant, 1995). pH is an outcome as well as an important determinant of rumen function. Few attempts have been made to model rumen pH and most models consider pH as an input or use a rather empirical approach to estimate it (Argyle & Baldwin, 1988; Lescoat & Sauvant, 1995; the empirical option of Pitt *et al.*, 1996; Mills *et al.*, 2001). Only a few examples are available of modelling pH in a more mechanistic manner (Pitt *et al.*, 1996; Dunlap & Kohn, 1998; Imamidoost & Cant, 2005). In a recent study by Bannink & Dijkstra (2006) a modelling approach was used to integrate various factors that affect acid-base equilibrium in the rumen. Factors included were VFA, phosphate, ammonia, cations and anions, partial CO₂ gas pressure, and cation-anion exchange capacity of feed substrate. The results confirm the conclusions by Counotte (1981) and other modelling exercises that, after the clearance of VFA, saliva secretion is the second most important buffering mechanism of rumen acidity. Some rumen models consider saliva production (Baldwin *et al.*, 1987; Baldwin, 1995; Pitt *et al.*, 1996; Dijkstra *et al.*, 1992) but attempts to predict its effect on rumen pH have been limited. For prediction of the effect of feeding strategy on fluctuation in rumen pH during the day, the effects of feed intake pattern on the moment and rate of saliva production deserve more attention. The amount of saliva secreted may be several hundreds of litres per day in a lactating cow (Maekawa *et al.*, 2002) and depends on dietary factors that affect the rate at which a meal is consumed and the time spent eating and ruminating. More fibrous meals or meals of a larger particle size are eaten more slowly and hence result in more saliva production, whereas rumen contents appear less important (Bailey, 1961; Bailey & Balch, 1961). However, Maekawa *et al.* (2002) were not able to reproduce these results and found that increased saliva production with an increased time spent eating and ruminating was compensated by decreased secretion with a decreased time spent resting. More research seems necessary to clarify the situation for lactating cows under various nutritional treatments. Simultaneously, effects on rumen water dynamics need to be included because they also

affect concentrations of acids and bases present in rumen fluid (Baldwin, 1995; López *et al.*, 2003). Despite the important buffering effects of saliva, accurate prediction of rumen pH first of all requires a reasonable prediction of rumen VFA concentrations, and consequently of the process of VFA absorption and VFA outflow in rumen fluid. Clearance of rumen VFA is the main buffering mechanism and in this respect the interactions with rumen wall functionality are a crucial aspect to consider. In one dynamic and one static modelling effort, the process of VFA absorption was addressed in an empirical manner (Dijkstra *et al.*, 1993; Pitt *et al.*, 1996; Chapter 7). All other modelling efforts represent intra-luminal processes without giving much appraisal of the cow surrounding the lumen.

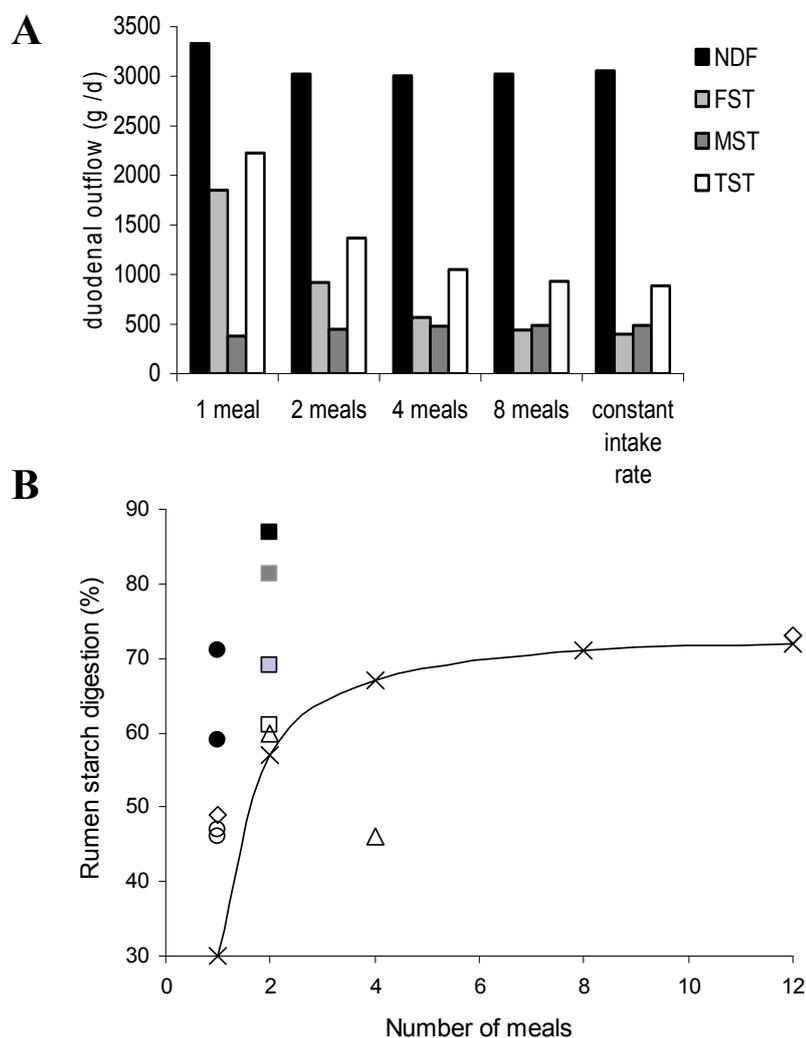


Figure 3. (A) Predicted effects of the number of meals of equal composition on duodenal flow of NDF, feed starch (FST), microbial starch (MST) and total starch (TST) (Bannink & Dijkstra, 2007); (B) Observed effects *in vivo* of the number of meals on rumen starch digestion of ground corn (open symbols) or rolled corn (hatched symbols), and of ensiled corn with a low DM-content (closed symbols). Results were derived from Oba & Allen (2003, \circ), Knowlton *et al.* (1998, \square), Shabi *et al.* (1999, \triangle) and Froetschel & Amos (1991, \diamond). Results of Shabi *et al.* (1999) were based on abomasal sampling instead of duodenal sampling. The curve ($- \times -$) represents the simulation results depicted in (A).

Rumen VFA production

All models of whole rumen function use stoichiometric coefficients for the conversion of substrate to a certain type of VFA. The stoichiometry of Baldwin *et al.* (1970) was applied by Danfær (1990), and that of Murphy *et al.* (1982) by Baldwin *et al.* (1987) and Dijkstra *et al.* (1992). Since 1982, pH dependency has been introduced by Argyle & Baldwin (1988) based on *in vitro* data (Chapter 5). Pitt *et al.* (1996) derived an alternative stoichiometry from *in vitro* data. Following type of substrate, lactate and pH were introduced as important determinants of the pattern of VFA. Friggens *et al.* (1998) derived empirical equations relating feed characteristics to observed ruminal VFA in sheep. Nagorcka *et al.* (2000) derived a new stoichiometry from studies on microbial cultures and included a distinct stoichiometry for protozoa. Bannink *et al.* (2000; Chapter 6) derived a stoichiometry from data on lactating cows only. This latter stoichiometry has recently been evaluated against independent data on lactating cows (Bannink & Tamminga, 2005) and improved the prediction of VFA molar proportions (Bannink *et al.*, 2000; Chapter 6). A likely reason for this is that this stoichiometry was derived from actual *in vivo* measurements of rumen digestion and VFA molar proportions in the target animal, in contrast to all the other attempts. Still, a large fraction of observed variation remained unexplained.

More recently, Sveinbjörnsson *et al.* (2006) elaborated further on the approach of Bannink *et al.* (2000) but used different substrate classes (dietary contents of protein, lactic acid, forage NDF, concentrate NDF, starch and other OM, instead of digested amounts of soluble carbohydrate, starch, hemi-cellulose, cellulose and protein), and included the level of dry matter intake and concentrate ether extract as additional explanatory factors. Their approach was evaluated to give a reasonable prediction, but it is less related to the actual rumen fermentation conditions met and was evaluated on a rather narrow subset of the full dataset used to derive the coefficients (Dijkstra *et al.*, 2007a). The present study (Chapter 7) aimed to introduce pH dependency into the stoichiometric model, taking the effects of (observed or estimated) fractional fluid passage rate, rumen fluid volume and VFA absorption rate into account. In the data set on rumen starch digestion given in the review by Mills *et al.* (1999), the effect of rumen pH on propionate (Pr) yield from starch can be clearly seen. Regression results obtained in the present study indicate that a reduction in pH from 6.5 to 5.5 results on average in a 100% and 50% increase in the amount of Pr formed per unit of soluble carbohydrates and starch, respectively, fermented in the rumen (Bannink *et al.*, 2007). This result generally confirms earlier findings by Argyle & Baldwin (1988), though logistic rather than linear relationships with pH were needed to obtain satisfactory results, and *in vivo* data from lactating cows were used instead of *in vitro* data. Because Pr is the main precursor of glucose synthesis in the liver, this effect of rumen pH needs to be taken into account in nutrient based feed evaluation. Together with the effect of the intake pattern of starch-rich meals on starch degradation, and the substantial contribution of microbial starch to total starch

outflow to the duodenum with low-starch diets (discussed in the previous section), explanation of glucose supply to the cow probably requires evaluation of these three factors in an integrated manner.

Current relationships (Chapter 7) are thought to give a reasonable indication of the effects of type of substrate (associated with type of fermenting micro-organism), type of diet (fraction of concentrates in dry matter) and rumen pH on VFA yield (Dijkstra *et al.*, 2007a). Ideally, these relationships should be further evaluated with a set of *in vivo* observations of comparable size, but with a focus on variation in rumen pH or other rumen fermentation characteristics instead of testing sources of soluble carbohydrate or starch. Such a study would verify the applicability of the present relationships. Furthermore, the stoichiometry of VFA production had to be derived indirectly from rumen concentrations of VFA instead of VFA production rates, and several assumptions on rumen conditions had to be made (Bannink & Dijkstra, 2005). A major step forward would be the access to direct measurements of VFA production rates (Resende *et al.*, 2006; Sutton *et al.*, 2003) instead of rumen concentrations. Although more theoretical considerations such as thermodynamic aspects of the stoichiometry of VFA produced (Kohn & Boston, 2000; Offner & Sauvant, 2006) may also help in explaining changes in rumen VFA production, it must be ensured that results mirror the effects observed *in vivo* in the rumen of lactating cows. It is likely that the representation of the stoichiometry of VFA yield derived in the present study can be improved further when appropriate data become available. For example, the effect of protozoal activity on the type of VFA formed (Williams & Coleman, 1997) may be included in future analyses of the stoichiometry of VFA yield. Inclusion of the effect of protozoa was hampered in the present study because insufficient *in vivo* observations on rumen digestion, protozoal activity and VFA molar proportions were available, whereas *in vitro* data were considered inadequate.

The effect of protozoal activity on VFA yield was reviewed by Nagorcka *et al.* (2000) who estimated that 50% of the fraction of rapidly fermentable and structural carbohydrates converted into VFA leads to butyrate (Bu) formation, whereas no Pr is formed. If this protozoal stoichiometry applies, a substantial effect of changes in the protozoal population on molar proportions of Bu must be expected. In support of this, analysis of the effect of defaunating the rumen (Eugène *et al.*, 2004) indeed indicated that molar proportions of acetate (Ac), Pr and Bu changed by the order of -0.015, +0.030 and -0.015, respectively. The effects of protozoal activity on VFA are strongly confounded with other concomitant changes, however, which cause changes in the type of VFA produced as well. The effects on VFA established by Eugène *et al.* (2004) were associated with substantially (6% units) lower fibre digestion, and up to 0.3 units higher and lower rumen pH with low- and high- concentrate diets, respectively. Such effects also influence Bu molar proportions. For example, a 42% reduction in protozoal count with an increase in the amount of steam-rolled barley grain in the diet of steers to 90% of DM, resulted in only a slight decrease of Bu molar proportion (Hristov *et al.*, 2001). A large part of this effect on Bu may also be attributed to the

concomitant decrease in pH from 6.5 to 6.0, the relatively low Bu yield from starch with concentrate-rich diets (Chapter 7), the increase in dietary starch at the expense of NDF and soluble carbohydrates, or the reduced NDF degradation with lower pH. Another study with an (insignificant) numerical increase in protozoal count of 60% when a forage diet for heifers was supplemented with ground barley (Piwonka *et al.*, 1994) showed a slight increase in Bu by 2 mol/100 mol VFA and a decrease in pH post feeding by 0.2 units to 6.4.

Hence, it seems that consequences of changes in protozoal population size on VFA molar proportions remain rather small under normal physiological conditions. Although selective production of Bu and Ac by protozoa undoubtedly exists, larger effects on VFA are probably to be expected from exchanging different types of carbohydrates and from changes in rumen pH. Regressions results in the present study (Chapter 7; Bannink & Dijkstra, 2005) indicate that with substitution of soluble carbohydrates for starch, on average, 2.5 and 1.3 times as much Bu is generated at pH 5.0 and at pH 6.5, respectively (1.9 and 1.0 with forage-rich diets, and 3.0 and 1.6 with concentrate-rich diets, respectively). These estimates appear close to rumen observations without a numerical increase of protozoal count in dairy cattle (Hristov & Jouany, 2005). Supplementing an alfalfa hay diet for dairy cattle with 20% glucose instead of starch and fibre almost doubled the Bu molar proportion (0.018 versus 0.011 and 0.09) and decreased rumen pH (6.0 versus 6.2 and 6.4). The regression results also predict a small effect of the dietary changes on Bu yield as was reported in the study by Piwonka *et al.* (1994).

It is concluded that stimulation of protozoa adds to the formation of Bu, but that this effect is normally of smaller magnitude than the effects of type of substrate fermented into VFA, rumen pH and type of diet consumed. Nagorcka *et al.* (2000) discriminated between the stoichiometry of VFA production by bacteria and protozoa, but all of other effects were neglected. Notwithstanding potential improvements that can be made in future, the current representation of VFA stoichiometry is thought to give a reasonable explanation of VFA yields for the varying fermentation conditions met in the rumen.

Functionality of the rumen wall

There is much evidence that rumen epithelial tissues perform their functions at a relatively high rate and with high energy expenditure. *In vivo* measurements indicate that they account for 10% of total oxygen consumption by the whole body and about half of that by the portal drained viscera, that their rate of oxygen consumption per unit of tissue weight is about 7 times larger than that of the whole body, and that their function to absorb VFA delivers up to two thirds of the total energy absorbed from the gastrointestinal tract (Huntington & Reynolds, 1987). Further, there is evidence of intensive adaptation of the morphology of the rumen wall during the transition period and in early lactation, which is of primary importance

to the performance of the dairy cow (Dirksen *et al.*, 1984; Bannink *et al.*, 2005c, d). Much more information is available from *in vitro* studies on the transport of solutes (Rechkemmer *et al.*, 1995; Gäbel, & Sehested, 1997) and on metabolic activity in isolated sheets or samples of rumen epithelium (McBride & Kelly, 1990; Milligan & McBride, 1985) than from *in vivo* studies. Still, only a limited number of reports is available on enzymatic capacity of VFA metabolism in isolated rumen tissues (Ash & Baird, 1973; Harmon *et al.*, 1991; Scaife & Tichivangana, 1980) and in epithelial cell cultures (Baldwin & McLeod, 2000), despite the fact that VFA are the main energy source for these tissues (Rémond *et al.*, 1995). Most *in vivo* studies on VFA metabolism involved measurement of net portal appearance of intra-ruminal infusions of VFA. Such data reflect the metabolic activity of the whole package of portal drained viscera, however, which complicates their use for rumen modelling. For example, Kristensen & Harmon (2004a, b) demonstrated that rumen tissues in steers hardly contributed to the intensive metabolism of Ac by portal drained viscera, which is in contrast to earlier views on rumen VFA metabolism (Bergman, 1990; Seal & Reynolds, 1993). These results indicate that measurements of net portal flux of VFA alone are insufficient to explain variation in metabolism of rumen tissues and in metabolism of these nutrients. With respect to this limitation, Reynolds *et al.* (1994) concluded that the metabolic region between the lumen of the gut and the gastrointestinal capillaries need further and innovative research. Still, today hardly any study is available which combines measurement of rumen fermentation processes on the one hand (microbial activity, substrate degradation, VFA production) and functionality of the rumen wall with undisturbed rumen contents (VFA absorption, intra-epithelial VFA metabolism, epithelial development and morphology) on the other.

Transport of VFA across the rumen wall is the most important mechanism for preventing build-up of acids in rumen contents. Along with VFA transport, there is extensive transport of ions (Gäbel & Sehested, 1997; Bannink & Tamminga, 2005). The other main mechanism for buffering rumen acidity is saliva secretion, which has already been discussed. Microbial fermentation, buffering mechanisms, and rumen wall functioning and metabolism only become apparent in a strongly confounded manner with *in vivo* observations. Hence, delineating the contribution of these various processes to whole rumen function remains difficult without adopting a modelling approach (Chapter 7). Whereas all rumen models address variation in microbial activity and substrate degradation in response to dietary characteristics, attention to the functionality of the rumen wall is sparse. Most rumen models represent recycling of urea-N from blood to the rumen when N availability in the rumen is low. Some models represent variation in fractional absorption rates of VFA, but further information on rumen wall functioning is essentially lacking. It is concluded that the latter is needed, to some extent at least, because whole rumen function not only depends on intra-luminal processes but also on those between the lumen and the capillaries (Reynolds *et al.*, 1994).

Interactions between the production, absorption and metabolism of VFA

The main VFA formed in the rumen are Ac, Pr and Bu. Reported fractional rates of VFA absorption from the rumen generally increase with VFA chain length, and the differences in absorption rates between individual VFA vary between 5 and 50% (Kristensen & Harmon, 2004b; Kristensen *et al.*, 2000; López *et al.*, 2003; Dijkstra *et al.*, 1993). Besides methodological differences, different testing conditions with respect to VFA concentrations, pH, fluid volume, fluid osmolarity, arterial VFA concentrations, blood flow and the state of rumen tissues likely affect differences in results between various studies. Fractional rates of VFA absorption from the rumen are thought to be sensitive to concentration gradients between the intra-luminal, intra-epithelial and arterial blood compartments (Dijkstra *et al.*, 1993; Kristensen & Harmon, 2004b; Chapter 7). Next to VFA production rates, epithelial mass and epithelial metabolic activity are expected to determine the concentration gradients between rumen, epithelium and blood. Different explanations may be given for observed VFA absorption rates with various VFA infusion protocols or rumen VFA mixtures.

For example, Kristensen *et al.* (2000), from an *in vivo* study with intra-ruminal VFA infusion in sheep, concluded that the interaction between VFA and their activation of CoA-synthetases established in *in vitro* studies was not reflected in their results. However, no satisfying explanation was given for the extreme changes and differences in fractional absorption rate of individual VFA when Bu was substituted for Ac in the VFA mixture incubated. Change of VFA composition of the rumen infusate (from 63% Ac and 6% Bu to 17% Ac and 42% Bu) resulted in a disproportionately small decrease in rumen Ac concentration (from 68 to 46 mol/100 mol VFA) and an increase in Bu concentration (from 6 to 23 mol/100 mol VFA). In another study using an increasing rate of Bu infusion and a corresponding decreasing rate of Ac infusion into the emptied and washed rumen of steers, fractional absorption rate of Bu decreased when the molar proportion of Bu in the infusate was increased to 0.36, leading to a rumen Bu concentration of 37 mmol/l (Kristensen & Harmon, 2004b). It is interesting to note that the concomitant increase in fractional absorption rate of Ac was 19%, whereas that of Pr remained unaffected. These results may be explained by a change of the intra-epithelial concentration of Ac and Bu and the resulting shift in the rates of Ac and Bu activation towards less and more saturated levels, respectively, which affect rumen/intra-epithelial concentration gradients and absorption rates. Results of Krehbiel *et al.* (1992), who infused increasing amounts of Bu into the undisturbed rumen of steers, support this explanation. A more than proportional increase in rumen Bu concentration started to appear at 28 mmol/l, which indicates a reduced fractional absorption rate. No change in fractional absorption rate became apparent for rumen Ac. In contrast to an almost halved Ac concentration in the experiment of Kristensen & Harmon (2004b), rumen Ac concentration was reduced only slightly from 59 to 55 mmol/l with the highest Bu infusion and may have been associated with a reduction in pH of 0.3 units to 6.4 (Dijkstra *et al.*, 1993).

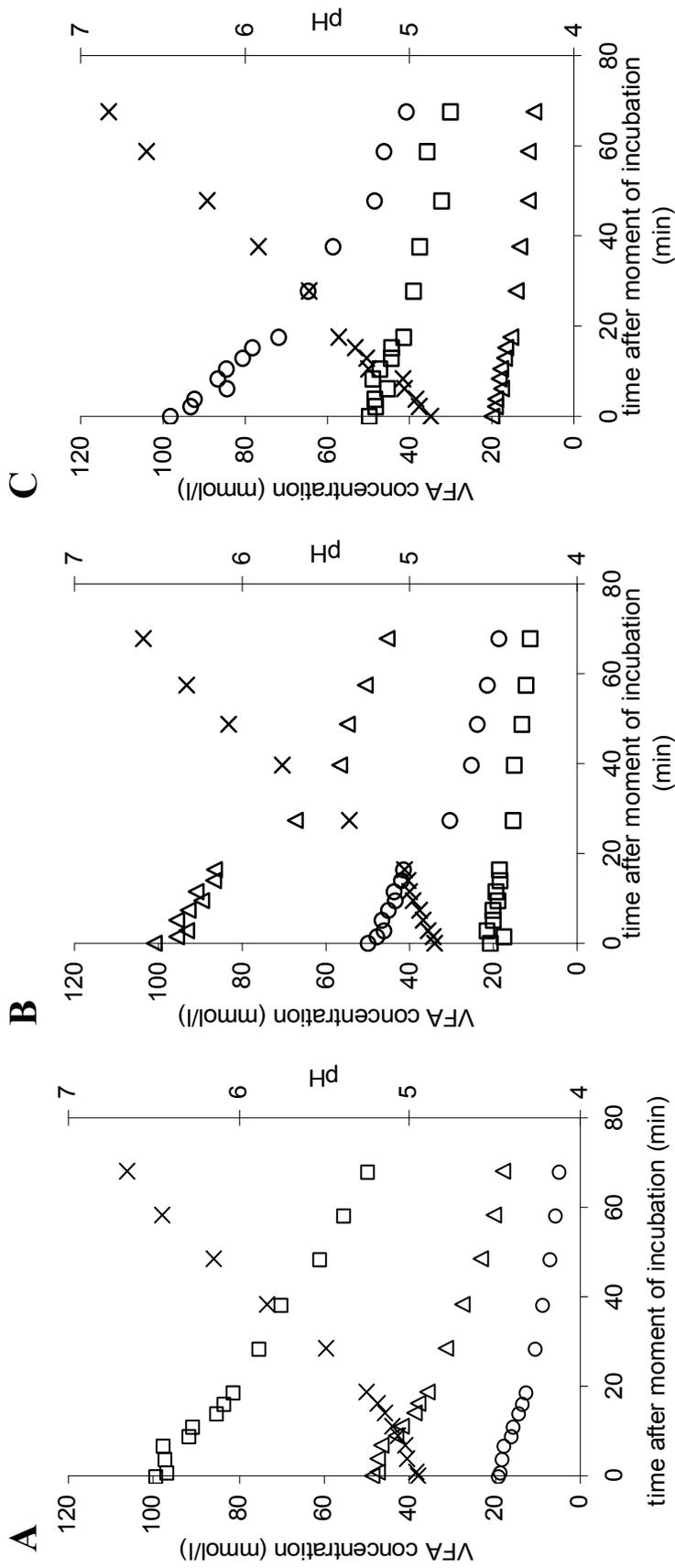


Figure 4. Acetate (\square), propionate (\triangle) and butyrate (\circ) concentrations and pH of rumen fluid, after introduction of a VFA buffer of 40 liters into an emptied and (with VFA buffer) washed rumen. The VFA buffer contained (A) 100, 50 and 20 mM, (B) 20, 100 and 50 mM, and (C) 50, 20 and 100 mM of acetate, propionate and butyrate, respectively.

Table 1. Clearance of VFA from the rumen and the fraction not recovered in the net portal flux of VFA during 70 minutes following the incubation of experimental solutions containing various mixtures of VFA and Co-EDTA in an emptied and washed rumen of a lactating dairy cow consuming daily 18 kg of dry matter of maize silage and grass silage in the proportion of 2:1 (unpublished results).

	100 : 50 : 20		20 : 100 : 50		50 : 20 : 100	
	start	end	start	end	start	end
pH rumen fluid	5.0	6.7	4.9	6.6	4.9	6.8
Volume rumen fluid (L) ¹	40.0	38.0	40.0	43.7	39.8	39.3
Fluid outflow rate (%/h) ²	25.8		29.3		32.4	
Clearance of rumen VFA (%) ³						
Ac	52		41		40	
Pr	65		51		51	
Bu	77		59		59	
Total VFA	59		52		53	
Absorbed VFA not recovered in portal net flux (%) ⁴						
Ac	48		89		86	
Pr	19		56		58	
Bu	71		78		69	
Arterial concentration (mM) ⁵						
Ac	2.09		0.71		1.19	
Pr	0.31		0.55		0.18	
Bu	0.18		0.21		0.87	
β -OH-Bu	0.63		0.94		1.95	
La	0.91		1.16		0.53	

¹ End volume was estimated by the evacuation of rumen content at the end of the incubation period.

² Estimated from the decline in Co-EDTA concentration measured at the start and the end of the trial.

In contrast to the findings in steers, no saturation of Bu absorption was established by Dijkstra *et al.* (1993) in lactating cows but it became apparent for Ac in particular. The highest Bu concentration in their experiment was 100 mmol/l. This result may be an indication that different characteristics of VFA transport and intra-epithelial metabolism exist between sheep, steers and dairy cows. On the other hand, some further results from our own laboratory on a lactating dairy cow (Table 1; unpublished results) did indicate that clearance of Bu may saturate at very high rumen concentrations (up to 100 mmol/l). Three different VFA mixtures were introduced into the emptied and washed rumen, and clearance of VFA, water outflow and net portal fluxes of VFA, β -OH-Bu and lactate were measured. With all mixtures, clearance always increased in the order of Ac, Pr and Bu, which corresponds to previous findings (Dijkstra *et al.*, 1993). However, clearance of Bu was reduced by 23% with the VFA mixtures containing 50 and 100 mmol Bu/l compared to that containing 20 mmol/l. This corresponds to the saturation of Bu clearance established at rumen concentrations of 28 and 37 mmol/l by Krehbiel *et al.* (1992) and Kristensen & Harmon (2004b). The effect of Bu concentration was confounded, however, with those of Ac and Pr concentration. With 50 mmol Bu/l, the extremely high Pr concentration of 100 mmol/l might have inhibited Bu metabolism and absorption, whereas with 100 mmol Bu/l a high intra-epithelial Bu concentration might have saturated its metabolism (Table 1). To a similar extent (21%), clearance of Ac was probably reduced by inhibition of its metabolism with 50 and 100 mmol/l of Pr or Bu. Clearance of Pr was reduced (22%) because its metabolism was inhibited with 100 and 50 mmol Bu/l, or saturated with 100 mmol Pr/l. Seal & Parker (1994) applied increasing rates of intra-ruminal Pr infusion to steers but their results remained inconclusive in this regard because the maximum rumen concentration achieved was 25 mmol/l, which is close to the lowest level tested in our experiment. It is concluded that *in vivo* results generally confirm the concept that inhibition or saturation of VFA activation rate in rumen epithelia may lead to intra-epithelial accumulation of VFA in epithelial tissues, a reduced rumen/intra-epithelial concentration gradient and, consequently, a reduced VFA transport rate and clearance from the rumen. The highest rate of VFA clearance was established with the VFA mixture closest to normal physiological conditions (high molar proportions of Ac and low molar proportions of Bu) (Table 1). This finding is supported by Kristensen *et al.* (2000) who maintained similar rumen VFA concentrations with the most realistic VFA mixture in spite a 25% higher rate of VFA infusion.

Careful inspection of the results of our experiment indicates some further interesting phenomena. Figure 4 illustrates the time course of rumen VFA concentrations and pH during the absorption trials. According to the concepts described in Chapter 7, a distinction has to be made between diffusion of undissociated VFA and facilitated transport of dissociated VFA. As fluid pH at the start of the trial was similar to the pKa of VFA, both forms of VFA accounted for about 50% of total VFA concentration. In correspondence with the assumptions made in Chapter 7 on fractional rate constants for passive diffusion, the initial rates of

clearance at the start of the trials were larger in the order Ac, Pr, Bu (Figures 4A, 4B & 4C, respectively). During this initial phase, VFA clearance appears rate-limited by passive diffusion rather than facilitated transport, and pH gradually increased to 5.1 (duration about 7, 15 and 10 minutes, respectively, in Figures 4A, 4B & 4C). After this phase, VFA clearance accelerated and pH increased faster. During this second phase the rate of VFA clearance was probably rate-limited by facilitated transport rather than passive diffusion. Interestingly, no distinction between the initial and second phases became apparent for Bu clearance with the VFA mixture containing most Bu (Figure 4C). A likely explanation is that the rate of passive diffusion closely matched that of facilitated transport, masking a distinction between the two phases.

The mixture which contained most Ac showed the highest fraction cleared from the rumen at the end of the trial (Table 1), despite the fact that Ac has the lowest fractional diffusion rate (Figure 4A). This can be explained by the fact that Bu concentration was lowest in this particular mixture, and hence least inhibition of intra-epithelial Ac and Pr activation occurred, as already discussed. Although water flows to and from the rumen may also have been responsible for different extents of VFA clearance, this does not seem to have occurred because the highest clearance of VFA was associated with the lowest fractional outflow rate of water (Table 2). López *et al.* (2003) established in sheep that rate of water outflow increased with VFA concentrations up to 80 mmol/l. If this finding with sheep is valid for our study, the differences in VFA clearance during the initial phase with very high VFA concentrations of 170 mmol/l were not related to differences in water flows. The highest fractional rate of water outflow was observed for the VFA mixtures that cleared slowest (Table 1) and may have been related to the higher VFA concentrations established for these mixtures at the end of the second phase. Regardless of the precise cause, higher rates of water outflow inflated clearance of VFA with these mixtures, masking the slower absorption of VFA by the rumen wall.

In conclusion, interpretation of observations on rumen VFA absorption requires acknowledgement that apparent absorption is the outcome of intricate interplay between ruminal production of individual VFA, VFA transport across the epithelium via different routes, rumen water dynamics, and intra-epithelial metabolism.

Characterisation of intra-epithelial VFA activation

In the previous section, the importance of intra-epithelial activity of CoA-synthetases was discussed. In the present study (Chapter 7) the choice was made to parameterize representation of VFA metabolism by the rumen wall with data from Harmon *et al.* (1991), although these were obtained in calves rather than dairy cows. Though less complete, the data of Ash & Baird (1973) from mature non-lactating Friesian x Ayrshire cows may be more representative for the cow as the target animal. The reciprocal plots in Figure 5 (Bannink *et al.*, 2000; Bannink *et al.*, 2006b) demonstrate the differences in outcome of both studies.

Although a similar mechanism of competitive inhibition among individual VFA became apparent, the level of CoA-synthetase activity for Ac was much lower in the dairy cows. Table 2 shows the sensitivity of model predictions against the size of difference in CoA-synthetase activities established in both studies. Use of mature cow data resulted in a significant reduction in predicted Ac and Pr metabolism, more in line with the finding of Kristensen & Harmon (2004a) that there is hardly any contribution from rumen epithelia to the intensive metabolism of Ac in the portal drained viscera of steers. Although Kristensen (2005) suggested this to be a general phenomenon of rumen VFA metabolism, it still remains to be confirmed by other studies. It may be questioned for lactating cows with much higher levels of feed intake, drastic changes during the period of late gestation and early lactation, and higher metabolic activity of rumen epithelial tissues. The tissues undoubtedly have the enzymatic capacity to metabolize Ac (Figure 5) which can probably be utilized to a variable extent, depending on the physiological functions these tissues have to maintain, the energy and nutrient requirements associated with them, and the nutrients supplied by the rumen and arterial blood.

The sensitivity of model outcomes to CoA-synthetase activities indicates that more conclusive data are needed on these enzyme activities in response to variation in activity, development and morphology of rumen epithelia that occurs with changes in feeding strategy in lactating cows. Preferably, such data are gathered in combination with *in vivo* measurements of VFA absorption and metabolism.

In conclusion

Apparent discrepancies between *in vivo* data on VFA absorption, metabolism and portal net appearance, and *in vitro* data on VFA activation rates and energy requirements by rumen tissue should not immediately lead to rejection of the concepts proposed. Besides apparent metabolism of VFA by the portal drained viscera, specific attention to the interrelationships between them is needed. From the perspective of published *in vivo* results as well as from the modelling one in the present study (Chapter 7), there are indications of competitive inhibition among individual VFA with both rumen absorption and intra-epithelial metabolism of VFA. In combination with the pronounced adaptation of rumen epithelia to feeding strategy and nutrient supply, the role of the rumen wall is probably more important in quantifying rumen function and nutrient absorption from the gastrointestinal tract than is generally recognized. In particular, experimental work is needed on rumen wall functioning with lactating cows as the target animal rather than steers or sheep.

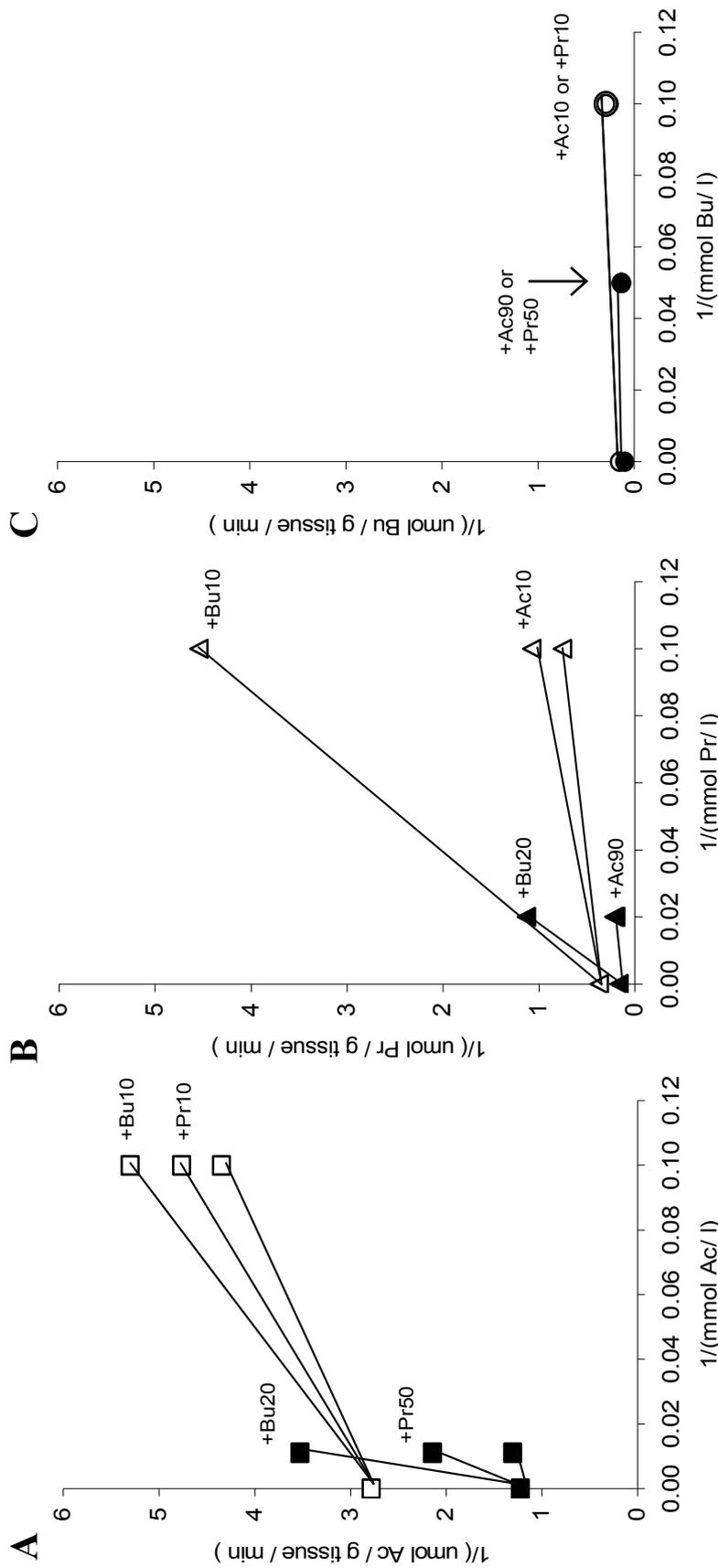


Figure 5. Comparison of enzyme assays in rumen epithelium by Harmon *et al.* (1991; closed symbols) and Ash & Baird (1973; open symbols). The effect of inhibiting VFA on the activity of VFA activation is demonstrated by a double-reciprocal plot of Co-synthetase activity (in $\mu\text{mol VFA} / \text{g tissue} / \text{minute}$) and VFA-concentration of the activated VFA type (in mmol/l); (A) acetate, \square and Ac; (B) propionate, \triangle and Pr; (C) butyrate, \circ and Bu. Codes and numbers that guide the symbols indicate the type and concentration (in mmol/l) of inhibiting VFA (absence of a guiding code indicates absence of inhibiting VFA). Graphs are taken from Bannink *et al.* (2000) and Bannink *et al.* (2006b).

Table 2. Steady-state simulations¹ for the effect of VFA molar proportions on the extent of intra-epithelial VFA metabolism with the original parameterisation according to Harmon *et al.* (1991) (H; Chapter 6) and with an adapted parameterisation according to Ash & Baird (1973) (A).

Data used for parameterization	Extent of intra-epithelial metabolism ²					
	Ac		Pr		Bu	
	H	A ³	H	A ³	H	A ³
Molar proportion rumen VFA Ac : Pr : Bu (mol / 100 mol VFA)						
70 : 15 : 15	13.5	6.9	32.9	12.8	79.3	68.5
50 : 35 : 15	14.2	7.0	30.3	12.3	79.1	68.5
60 : 35 : 5	14.2	6.8	35.7	16.5	81.3	71.5

¹ Model inputs and parameters not mentioned here were kept identical to those mentioned in Table 2 of Chapter 6.

² Percentage of the VFA produced in the rumen that does not appear in portal blood; Ac, acetate; Pr, propionate; Bu, butyrate.

³ Original estimates of parameter values for enzyme kinetics of intra-epithelial metabolism derived from results of Harmon *et al.* (1991) in calves were re-estimated from results of Ash & Baird (1973) in mature cows, illustrated in Figure 4. The maximum velocity of VFA activation by CoA-synthetases was re-estimated at 50%, 50% and 66% of the original value in the case of Ac, Pr and Bu; inhibition constants for the inhibition of Ac activation by Pr and Bu were re-estimated at 100% and 200% of the original value, for the inhibition of Pr activation by Ac and Bu at 20% and 50% of the original value, and for inhibition of Bu activation by Ac and Pr both at a much smaller value of 0.2 mol/l.

Future rumen modelling

Since publication of the dynamic models studied in Chapters 2 to 4, several new aspects have been added to them. To the model of Dijkstra *et al.* (1992), a more refined representation of protozoal activity and protozoa–bacteria interrelationships (Dijkstra, 1994) and a representation of feed intake pattern were introduced (Chapter 5; Bannink & Tamminga, 2005; Bannink & Dijkstra, 2007). The stoichiometry of VFA production in the rumen has also been improved (Chapters 6 & 7; Bannink *et al.*, 2000; Bannink & Dijkstra, 2005). The effects of fats and fatty acids on microbial activity have been added (Dijkstra *et al.*, 2000), and an integrated representation of nitrogen and phosphorus metabolism was introduced (Kebreab *et al.*, 2004). Factors affecting rumen pH have been studied (Bannink & Dijkstra, 2006) and empirical equations introduced which indicate whether a specific nutrient absorbed from the gastrointestinal tract (aminogenic, ketogenic or glucogenic) may be limiting milk synthesis (Dijkstra *et al.*, 1996, 2007c; Bannink & Dijkstra, 2007). Effects on methane yield were included (Mills *et al.*, 2001; Bannink *et al.*, 2005a, b; Dijkstra *et al.*, 2007b) and finally, a representation of epithelial development, VFA transport routes and intra-epithelial VFA metabolism has been added (Chapter 7).

The model of Danfær (1990) has not been developed further. To the model of Baldwin *et al.* (1987), the effect of pH on cell wall degradation and VFA production, and rumen water dynamics (Argyle & Baldwin, 1988; Baldwin, 1995) were incorporated. Recently, intrinsic degradation characteristics have been introduced as additional model inputs (Hanigan *et al.*, 2006).

Extending the present study

A further aspect to be added to the model developed in the present study is the relationship between VFA supply, VFA activation rate and yield and requirements for ATP and NADP(H) to sustain various physiological functions of the rumen epithelia. High energy costs are involved with functions such as tissue growth and proliferation, tissue turnover, and several other maintenance aspects such as VFA and ion transport (Summers *et al.*, 1988; Baldwin, 1995) and processes maintaining intra-epithelial homeostasis (Enemark *et al.*, 2002; Bannink & Tamminga, 2005; Bannink *et al.*, 2006b). Furthermore, the model perhaps needs to be extended to include representation of arterial uptake of nutrients (VFA, glucose and amino acids), of the mechanisms involved in coping with high acid loads and of other factors that lead to proliferation of epithelia (Mroz *et al.*, 2005; Bannink *et al.*, 2006b). Observations by Reynolds & Huntington (1988a, b) illustrate the impact of VFA production rate on nutrient utilization by rumen epithelia in growing steers. Compared to a roughage diet, the concentrate diet gave a threefold higher net appearance of Bu in portal blood, accompanied by ten fold and six fold increases in net utilisation of amino acids and glucose by the stomach tissues. Also VFA utilisation seemed higher as total net appearance of VFA in the portal vein was lower despite a higher digestibility of the diet. Results of Seal *et al.* (1992) were less

conclusive but also showed the combination of a numerically higher net portal appearance of Bu and increased utilization of amino acids and glucose by stomach tissues on a forage diet compared to a forage-concentrate diet. Krehbiel *et al.* (1992) established a numerical but statistically non-significant 28% reduction in net appearance of Ac in portal blood with the highest intra-ruminal infusion rates of Bu, perhaps also reflecting increased metabolic activity and growth of stomach tissues.

A combination of experimental work and modelling work is proposed to address functionality of the rumen wall under various production conditions. Particular attention to the cumulative effect over time of feeding strategy on epithelial tissues is needed. Perhaps modern molecular techniques might prove helpful in describing the mechanisms involved with adaptation and functionality of rumen wall tissues, as long as these techniques are not too qualitative. To incorporate such data into rumen models in order to delineate the impact of nutritional, animal and environmental factors in a more integrated manner is a challenging task. In general far more data, and more precise data, are needed (based on hypotheses on epithelial function; Reynolds *et al.*, 1994) than the information available for the present study. *In vivo* work that tests hypotheses on the interaction between VFA production, VFA absorption and epithelial nutrient requirements and the consequences for nutrient availability is needed. When carried out in an undisturbed rumen, such studies are preferably combined with the measurement of actual VFA production rates by the use of stable isotope techniques.

Wider application of rumen models

Rumen models are research tools for addressing a much wider scope of questions. The importance of predicting cow performance goes beyond prediction of digestion and lactation performance, and can include several other aspects of dairy farming. Usually, such side-effects are predicted with empirical relationships or 'rules of thumb' under the assumption of general applicability. Sometimes predictions are based on calculation rules of the feed evaluation systems applied in current practice. But such approaches presume a certain lactation performance instead of predicting it, they do not take into account the interactions between different side-effects (e.g. the relationship between milk, excreta, ammonia and methane), and because of their empirical nature, they lack the capability of explaining the effects of various influencing factors in an integrated manner (i.e. according to an underlying mechanism). The effects of these factors become apparent in a strongly confounded manner, complicating their prediction by empirical approaches alone (Tamminga *et al.*, 2007b). An example is prediction of the risk of development of subclinical acidosis (Enemark *et al.*, 2002) in cows around parturition and in early lactation. Available literature often refers to this condition but it appears poorly defined, understood and diagnosed. Modelling efforts may help in elucidating the importance of the mechanisms that are involved with adaptation of the cow around calving and with regulation of rumen acidity. Critical loads of acid may be predicted, or feeding strategies aimed at preventing the development of acidosis may be evaluated.

Besides cow related issues, the models are useful for resolving environmental issues. The detailed representation in the models may generate a more precise understanding of effects on site and composition of excreta, composition and characteristics of N in cattle manure, ammonia emissions and fertilizing potential of manure (Dijkstra & Bannink, 2000; Reijs *et al.*, 2007), variation in milk urea as an indicator of nitrogen utilization (Bannink *et al.*, 2006a), phosphorus excretion (Kebreab *et al.*, 2004) and methane emissions (Mills *et al.*, 2001; Bannink *et al.*, 2005a, b; Dijkstra *et al.*, 2007b; Tamminga *et al.*, 2007b). Current feed evaluation systems (Tamminga *et al.*, 1994, 2007a) or other empirical approaches (Kebreab *et al.*, 2001; Schröder *et al.*, 2006) may be used to predict N excretion and ammonia emissions (Van Duinkerken *et al.*, 2005), but these do not consider, for example, variation in protein degradation with feed intake level, variation in microbial efficiency, or amount and type of VFA available to the cow. Because methane emission is coupled with VFA yield (Mills *et al.*, 2001; Bannink *et al.*, 2005a, b; Dijkstra *et al.*, 2007a) these empirical systems are not suitable for studying interactions between ammonia and methane emissions with varying feeding strategies. Also evaluations may be hampered because effects on rumen digestion cannot be distinguished from intermediary metabolism and the productive functions of the cow.

Dynamic, mechanistic rumen models may deliver the type of information needed to evaluate in detail the consequences of feeding strategies on the level of whole farm nutrient management. In particular the impact on N losses is relevant in this respect (Bannink *et al.*, 2006c). Mechanisms represented involve N fluxes to and from the rumen, and microbial response to variation in availability of protein and ammonia as N sources and of carbohydrates as the main energy source (Chapters 1 to 3). An important question to answer is, for example, the extent to which N is limiting microbial activity and whether supplementing N in the diet will improve microbial protein synthesis or add to N loss from the rumen and subsequently from the cow through urine excretion (Dijkstra & Bannink, 2000). Recent simulations demonstrated that with an increasing amount of maize silage substituting grass silage (with a fixed 20% standard concentrate in the dietary DM), saturation of the rate of N recycling from blood to the rumen appeared at 60% maize silage in dietary DM. This result suggests that fairly low dietary crude protein contents of 12% DM may be possible without loss of ruminal fermentative capacity. Such results were observed for dairy cows in the study of Klusmeyer *et al.* (1990) in which even 11% crude protein in the dietary DM did not have a detrimental effect on microbial protein synthesis in the rumen. From the perspective of maintaining rumen fermentation capacity, there is therefore much potential to reduce further the protein content of the diet, N excretion rate, and ratios of mineral N:organic N and C:N in manure (extremes values differ by more than a factor of three; Reijs *et al.*, 2007; Bannink *et al.*, 2007). Precise evaluation of the changes in these aspects is important information for developing fertilization recommendations that take into account the long term effects of manure application (Schröder *et al.*, 2005). From the perspective of protein requirements of high-yielding cows, the options are probably more limited because, first, the

quantity and quality of forages needed puts limits on the possibility of reducing their N content. Second, metabolizable protein may become limiting for milk protein synthesis and maintenance functions, in particular during early lactation with cows in negative energy balance. Dynamic, mechanistic rumen models, in combination with improved estimates of requirements for glucose and protein for milk synthesis and maintenance functions, may aid in evaluating the consequences of feeding strategy on rumen function, N digestion and site of N excretion (urine versus faeces) as well as cow performance (Dijkstra *et al.*, 1996, 2007b).

In conclusion, the models are particularly useful for addressing the many dietary differences between farms, or between different production conditions or feeding strategies. They are more capable of evaluating extreme or atypical conditions, or when searching for the limits on nutritional measures (Bannink *et al.*, 2005a), such as lowering protein allowance to dairy cows, because of the mechanistic representation adopted rather than the use of empirical relationships.

Implications for feed evaluation

A major advantage of using dynamic rumen models is the number of factors that may be taken into account in an integrated manner. In a recent review on splanchnic VFA metabolism, Kristensen (2005) concluded that for practical use a large number of critical ruminal variables need to be predicted in order to describe satisfactorily the supply of individual VFA to the cow, interactions between nutrients, intermediary metabolism and productive functions.

Cows in early lactation have a large glucose demand, doubling within less than 2 weeks (Reynolds *et al.*, 2003). Balancing glucose supply with demand in response to feeding strategy and milk yield hence seems essential in any nutrient based feed evaluation for cows. The present study attempted to improve the prediction of the supply of ketogenic and glucogenic VFA from the rumen (Chapter 6) by introducing well-documented effects of pH, or associated rumen fermentation conditions, on the type of VFA formed from rapidly fermentable carbohydrates. Although there is extensive intra-ruminal conversion between individual VFA (Sutton *et al.*, 2003), this VFA stoichiometry is an estimate of the net yield of VFA taking into account the inter-conversions among individual VFA. Together with ruminal yield of Pr as the main precursor of gluconeogenesis in the liver, the dynamic rumen model of Dijkstra *et al.* (1992) also appears to provide a good basis for predicting the variation in rumen starch degradation, and supply of microbial starch and starch outflow to the intestine as a source of glucose for intermediary metabolism. The model already takes into account the variation in starch degradation depending on rumen conditions and variation in microbial starch synthesis. Recently the effect of number of starch meals on starch digestion has been added (Bannink & Dijkstra, 2007). Combining all these aspects allows prediction of the effect of various nutritional factors on glucose supply for intermediary metabolism. This prediction

will be an essential element of a nutrient based feed evaluation system. However it must also be noted that, while glucose supply may appear a primary determinant of milk production, under most nutritional conditions the capacity of glucose production in the cow is not limiting glucose supply (Huntington & Richards, 2005). Glucose synthesis by the liver is under tight metabolic control and dictated by glucose requirements (Reynolds *et al.*, 1994). As a result, predicting the response in milk yield to an increased supply of glucose must consider expected glucose demands. This demand depends on stage of lactation, potential for milk yield and supply of glucose and other nutrients to the udder. In their review, Nocek & Tamminga (1991) reported that milk yield was not affected by the additional glucose absorbed from the intestine, despite the large metabolic demand for glucose in a lactating cow.

It is concluded that dynamic rumen models have great potential for accommodating new findings from fundamental research and that they serve well as a basis for the development of nutrient based feed evaluation systems. Considering microbial activity and the digestive processes, these systems should also accommodate other aspects of rumen function which have been discussed in the present study. Although it will be a continuing challenge to translate and incorporate information from fundamental research into rumen and digestion models, extant models can be made applicable for practical purposes in two ways. First, they can serve to test concepts used in practice or they may be used to derive useful estimates or simplified relationships. Second, user interfaces can be developed which allow them to be applied more easily. With current Information and Communication Technology developments, the construction of such instruments is technically feasible, and hence attempts should be made to apply them in practice and for policy making and surveys, instead of keeping their use restricted to scientific and educational purposes.

References

- Argyle, J.L. & Baldwin, R.L., 1988. Modeling of rumen water kinetics and effects of rumen pH changes. *Journal of Dairy Science* 71, 1178-1188.
- Ash, R. & Baird, G.D., 1973. Activation of volatile fatty acids in bovine liver and rumen epithelium. *Biochemical Journal* 136, 311-319.
- Bailey, C.B., 1961. Saliva secretion and its relation to feeding cattle. 3. The rate of secretion of mixed saliva in the cow during eating, with an estimate of the magnitude of the total daily secretion of mixed saliva. *British Journal of Nutrition* 15, 443-451.
- Bailey, C.B. & Balch, C.C., 1961. Saliva secretion and its relation to feeding in cattle. 2. The composition and rate of secretion of mixed saliva in the cow during rest. *British Journal of Nutrition* 15, 383-402.
- Baldwin, R.L., Lucas, H.L. & Carbrera, R., 1970. Energetic relationships in the formation and utilization of fermentation end-products. In: A.T. Phillipson, E.F. Annison, D.G. Armstrong, C.C. Balch, R.S. Comline, R.S. Hardy, P.N. Hobson & R.D. Keynes (Eds.), *Physiology of Digestion and Metabolism in the Ruminant*. Oriel Press, Newcastle-upon-Tyne, United Kingdom, pp. 319-334.
- Baldwin, R.L., Thornley, J.H.M. & Beever, D.E., 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54, 107-131.
- Baldwin, R.L., 1995. Modeling Ruminant Digestion and Metabolism. Chapman & Hall Ltd, London, United Kingdom, p.578.
- Baldwin, R.L. & McLeod, K.R., 2000. Effects of diet forage:concentrate ratio and metabolizable energy intake on isolated rumen epithelial cell metabolism in vitro. *Journal of Animal Science* 78, 771-783.
- Bannink, A., Kogut, J., Dijkstra, J., France, J., Tamminga, S. & Van Vuuren, A.M., 2000. Modelling production and portal appearance of volatile fatty acids in cows. In: J.P. McNamara, J. France & D.E. Beever (Eds.), *Modelling Nutrient Utilisation in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 87-102.
- Bannink, A. & Tamminga, S., 2005. Rumen Function. In: J. Dijkstra, J.M. Forbes & J. France (Eds.), *Quantitative Aspects of Ruminant Digestion and Metabolism, 2nd Edition*. CAB International, Wallingford, United Kingdom, pp. 263-288.
- Bannink, A. & Dijkstra, J., 2005. Schatting van de vorming van vluchtige vetzuren uit gefermenteerd substraat in de pens van melkvee. Report 05/I002371 Nutrition & Food, Animal Sciences Group, Lelystad, The Netherlands (*in Dutch*).
- Bannink, A., Dijkstra, J., Mills, J.A.N., Kebreab, E. & France, J., 2005a. Nutritional strategies to reduce enteric methane formation in dairy cows. In: T. Kuczynski, U. Dämmgen, J. Webb & A. Myczko (Eds.), *Emissions from European Agriculture*, Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 367-376.
- Bannink, A., Dijkstra, J., Mills, J.A.N., Kebreab, E. & France, J., 2005b. A dynamic approach

- for evaluating farm-specific as well as general policies to mitigate methane emissions by dairy cows. *Proceedings 4th International Symposium on Non-CO2 Greenhouse Gases (NCGG-4). Science, Control, Policy & Implementation*. A. Van Amstel, Coordinator. Millpress (www.millpress.nl), Utrecht, The Netherlands.
- Bannink, A., Van Leeuwen, P.W., Stockhofe-Zurwieden, N., Valk, H., Hamminga, A.J. & Gerrits, W.J.J., 2005c. De invloed van de krachtvoerstrategie op de pensfunctie tijdens de lactatiestart. Report 05/I01034 Nutrition & Food, Animal Sciences Group, Lelystad, The Netherlands, p. 52 (*in Dutch*).
- Bannink, A., Van Leeuwen, P.W., Hamminga, A.J., Gerrits, W.J.J., Valk, H., Stockhofe-Zurwieden, N. & Van Vuuren, A.M., 2005d. The effect of feeding strategy after parturition on the development of rumen epithelium in dairy cows. In: *Proceedings European Society of Veterinary & Comparative Nutrition*, Turin, Italy.
- Bannink, A. & Dijkstra, J., 2006. Voorspelling van de zuurgraad van pensvloeistof. Internal ASG report 12, Animal Sciences Group, Lelystad, The Netherlands (*in Dutch*).
- Bannink, A., Dijkstra, J., Kebreab, E. & France, J., 2006a. Advantages of a dynamical approach to rumen function to help to resolve environmental issues. In: E. Kebreab, J. Dijkstra, J. France, A. Bannink & W.J.J. Gerrits, *Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches*. CAB International, Wallingford, United Kingdom, pp.281-298.
- Bannink, A., Dijkstra, J., Koopmans, S.J. & Mroz, Z., 2006b. Physiology, regulation and multifunctional activity of the gut wall, a rationale for multicompartmental modelling. *Nutrition Research Reviews* 19, 227-253.
- Bannink, A., Valk, H. & Mroz, Z., 2006c. Environmental preservation by adapting diets for dairy cows. In: R. Geers & F. Madec (Eds.), *Livestock Production and Society*. Wageningen Academic Publishers, Wageningen, The Netherlands, pp.151-165.
- Bannink, A. & Dijkstra, J., 2007. Toevoeging van enkele onderdelen aan een dynamisch pensmodel [weergave van voeropnamepatroon, vetzuurmetabolisme in de pens, en empirische vergelijkingen voor de voorspelling van darmvertering en melkproductie op basis van melksamenstelling]. Internal ASG report 72, Animal Sciences Group, Lelystad, The Netherlands (*in Dutch*).
- Bannink, A., Reijs, J. & Dijkstra, J., 2007. Integrated approaches to evaluate nutritional strategies for dairy cows. In: J. France & E. Kebreab (Eds.), *Mathematical Modelling in Animal Nutrition*. CAB International, Wallingford, United Kingdom, *in press*.
- Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70, 567-590.
- Casper, D.P., Maiga, H.A., Brouk, M.J. & Schingoethe, D.J., 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. *Journal of Dairy Science* 82, 1779–1790.
- Collao-Saenz, E.A., Bannink, A., Kebreab, E., France, J. & Dijkstra, J., 2006. Simulation of

- rumen particle dynamics using a non-steady state model of rumen digestion and nutrient availability in dairy cows fed sugarcane. In: E. Kebreab, J. Dijkstra, J. France, A. Bannink & W.J.J. Gerrits (Eds.) *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 33-39.
- Counotte, G.C.M., 1981. *Regulation of Lactate Metabolism in the Rumen*. PhD Thesis, University of Utrecht, Utrecht.
- Danfær, A., 1990. *A Dynamic Model of Nutrition Digestion and Metabolism in Lactating Dairy Cows*. PhD Thesis. Beretning fra Statens Husdyrbrugforsog 671. National Institute of Animal Science, Foulum, Denmark.
- Danfær, A., Huhtanen, P., Udén, P., Sveinbjörnsson, J. & Volden, H., 2006. The Nordic Dairy Cow Model, Karoline – Description. In: E. Kebreab, J. Dijkstra, J. France, A. Bannink & W.J.J. Gerrits (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 383-406.
- Dijkstra, J., Neal, H.D.StC., Beever, D.E. & France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *Journal of Nutrition* 122, 2239-2256.
- Dijkstra, J., 1993. *Mathematical modelling and integration of rumen fermentation processes*. PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M. & Tamminga, S., 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *British Journal of Nutrition* 69, 385-396.
- Dijkstra, J., 1994. Simulation of the dynamics of protozoa in the rumen. *British Journal of Nutrition* 72, 679-699.
- Dijkstra, J., France, J., Assis, A.G., Neal, H.D.StC., Campos, O.F. & Aroeira, L.J.M., 1996. Simulation of digestion in cattle fed sugarcane: prediction of nutrient supply for milk production with locally available supplements. *Journal of Agricultural Science* 127, 247-260.
- Dijkstra, J., France, J. & Davies, D.R., 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. *Journal of Dairy Science* 81, 3370-3384.
- Dijkstra, J. & Bannink, A., 2000. Analyses of modelling whole rumen function. In: M.K. Theodorou & J. France (Eds.), *Feeding Systems and Feed Evaluation Models*, CAB International, Wallingford, United Kingdom, pp. 299-322.
- Dijkstra, J., Gerrits, W.J.J., Bannink, A. & France, J., 2000. Modelling lipid metabolism in the rumen. In: J.P. McNamara, J. France & D.E. Beever (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 25-36.
- Dijkstra, J., Mills, J.A.N. & France, J., 2002. The role of dynamic modelling in understanding the microbial contribution to rumen function. *Nutrition Research Reviews* 15, 67-90.
- Dijkstra, J., Kebreab, E., France, J. & Bannink, A., 2007a. Modelling protozoal metabolism

- and VFA production in the rumen. In: J. France, E. Kebreab & B.W. McBride (Eds.), *Mathematical Modelling in Animal Nutrition*, CAB International, Wallingford, United Kingdom, *in press*.
- Dijkstra, J., Kebreab, E., Mills, J.A.N., Pellikaan, W.F., López, S., Bannink, A. & France, J., 2007b. Predicting the profile of nutrients available for absorption: from nutrient requirement to animal response and environmental impact. *Animal* 1, 99-111.
- Dijkstra, J., Kebreab, E., Bannink, A., Crompton, L.A., López, S., Abrahamse, P.A., Chilibroste, P., Mills, J.A.N. & France, J., 2007c. Comparison of energy evaluation systems and a mechanistic model for milk production of dairy cattle offered fresh grass-based diets. *Animal Feed Science & Technology*, (doi:10.1016/j.anifeedsci.2007.05.011), *in press*.
- Dirksen, G., Liebich, H.G., Brosi, G., Hagemesiter, H. & Mayer, E., 1984. Morphologie der pansenschleimhaut und fettsaureresorption beim Rind- bedeutende faktoren für gesundheit und Leistung. *Zentralblatt für Veterinärmedizin* A31, 414-430.
- Dunlap, T.F. & Kohn, R.A., 1998. Calculation of buffering capacity of bicarbonate in the rumen in vitro. *Journal of Animal Science* 76, 1702-1709.
- Enemark, J.M., Jorgensen, R.J. & Enemark, P.St., 2002. Rumen acidosis with special emphasis on diagnostic aspects of subclinical rumen acidosis: a review. *Veterinarija ir Zootechnika* 20, 16-29.
- Eugène, M., Archimede, H. & Sauvant, D., 2004. Quantitative meta-analysis of the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livestock Production Science* 85, 81-97.
- Firkins, J.L., Yu, Z. & Morrison, M., 2007. Ruminant nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy. *Journal of Dairy Science* 90, E1-E16.
- France, J., Thornley, J.H.M. & Beever, D.E., 1982. A mathematical model of the rumen. *Journal of Agricultural Science, Cambridge* 99, 343-353.
- Friggens, N.C., Oldham, J.D., Dewhurst, R.J. & Horgan, G., 1998. Proportions of volatile fatty acids in relation to the chemical composition of feeds based on grass silage. *Journal of Dairy Science* 81, 1331-1344.
- Froetschel, M.A. & Amos, H.E., 1991. Effects of dietary fiber and feeding frequency on ruminal fermentation, digesta water-holding capacity, and fractional turnover of contents. *Journal of Animal Science* 69, 1312-1321.
- Gäbel, G. & Sehested, J., 1997. SCFA transport in the forestomach of ruminants. *Comparative Biochemistry & Physiology. A. Physiology* 118, 367-374.
- Hanigan, M.D., Bateman, H.G., Fadel, J.G., McNamara, J.P. & Smith, N.E., 2006. An ingredient-based input scheme for Molly. In: E. Kebreab, J., Dijkstra, J., France, A., Bannink & W.J.J. Gerrits (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 328-348.
- Harmon, D.L., Gross, K.L., Krehbiel, C.R., Kreikemeier, K.K., Bauer, M.L. & Britton, R.A.,

1991. Influence of dietary forage and energy intake on metabolism and ayl-CoA synthetase activity in bovine ruminal epithelial tissue. *Journal of Animal Science* 69, 4117-4127.
- Huntington, G.B. & Reynolds, C.K., 1987. Oxygen consumption and metabolic flux of bovine portal-drained viscera and liver. *Journal of Nutrition* 117, 1167-1173.
- Huntington, G.B. & Richards, C., 2005. Metabolic fate of products of starch digestion and absorption in beef and dairy cattle. In: *Proceedings of the Southwest Nutrition & Management Conference*. Department of Animal Sciences, University of Arizona, Tucson, United States of America, pp. 67-77.
- Hristov, A.N., Ivan, M., Rode, L.M. & McAllister, T.A., 2001. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. *Journal of Animal Science* 79, 515-524.
- Hristov, A.N. & A.P. Jouany, 2005. Factors affecting the efficiency of nitrogen utilization in the rumen. In: A.N. Hristov & E. Pfeffer (Eds.), *Nitrogen and Phosphorus Nutrition of Cattle and Environment*, CAB International, Wallingford, United Kingdom, pp. 117-166.
- Imamidoost, R. & Cant, J.P., 2005. Non-steady-state modeling of effects of timing and level of concentrate supplementation on ruminal pH and forage intake in high-producing grazing ewes. *Journal of Animal Science* 83, 1102-1115.
- Jouany, J.P., Demeyer, D.E. & Grain, J., 1989. Effect of defaunating the rumen. *Animal Feed Science and Technology* 21, 229-265.
- Kebreab, E., Mills, J.A.N., Crompton, L.A., Bannink, A., Dijkstra, J, Gerrits, W.J.J. & France, J., 2004. An integrated mathematical model to evaluate nutrient partition in dairy cattle between the animal and its environment. *Animal Feed Science and Technology* 112, 131-154.
- Kebreab, E., France, J., Beaver, D.E. and Castillo, A.R. 2001. Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient Cycling in Agroecosystems* 60, 275-285.
- Kingston-Smith, A.H., Bollard, A.L., Thomas, B.J., Brooks, A.E. & Theodorou, M.K., 2003. Nutrient availability during the early stages of colonization of fresh forage by rumen micro-organisms. *New Phytologist* 158, 119-130.
- Klusmeyer, T.H., McCarthy, R.D., Clark, J.H. & Nelson, D.R., 1990. Effects of Source and Amount of Protein on Ruminal Fermentation and Passage of Nutrients to the Small Intestine of Lactating Cows. *Journal of Dairy Science* 73, 3526-3537
- Knowlton, K.F., Glenn, B.P. & Erdman, R.A., 1998. Performance, ruminal fermentation, and site of starch digestion in early lactation cows fed corn grain harvested and processed differently. *Journal of Dairy Science* 81, 1972-1984.
- Kohn, R.A., Boston, R.C., Ferguson, J.D. & Chalupa, W., 1994. The integration and comparison of dairy cows models. In: A. Danfaer & P. Lescoat (Eds.) *Proceedings of the IVth International Workshop on Modelling Nutrient Utilization in Farm Animals*. National Institute of Animal Science, Foulum, Denmark, pp. 117-128.

- Kohn, R.A. & Boston, R.C., 2000. The role of thermodynamics in controlling rumen metabolism. In: J.P. McNamara, J. France & D.E. Beever (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 11-24.
- Krehbiel, C.R., Harmon, D.L. & Schneider, J.E., 1992. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. *Journal of Animal Science* 70, 904-914.
- Kristensen, N.B., Pierzynowski, S.G. & Danfaer, A., 2000. Net portal appearance of volatile fatty acids in sheep intraruminally infused with mixtures of acetate, propionate, isobutyrate, butyrate, and valerate. *Journal of Animal Science* 78, 1372-1379.
- Kristensen, N.B. & Harmon, D.L., 2004a. Splanchnic metabolism of volatile acids absorbed from the washed reticulorumen of steers. *Journal of Animal Science* 82, 2033-2042.
- Kristensen, N.B. & Harmon, D.L., 2004b. Effect of increasing ruminal butyrate absorption on splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. *Journal of Animal Science* 82, 3549-3559.
- Kristensen, N.B., 2005. Splanchnic metabolism of volatile fatty acids in the dairy cow. *Animal Science* 80, 3-10.
- Lescoat, P. & Sauvant, D., 1995. Development of a mechanistic model for rumen digestion validated using duodenal flux of amino acids. *Reproduction, Nutrition et Development* 35, 45-70.
- López S., Hovell, F.D.D., Dijkstra, J. & France, J., 2003. Effects of volatile fatty acids supply on their absorption and on water kinetics in the rumen of sheep sustained by intragastric infusions. *Journal of Animal Science* 81, 2609-2616.
- Maekawa, M., Beauchemin, K.A. & Christensen, D.A., 2002. Effect of concentrate level and feeding management in chewing activities, saliva production, and ruminal pH of lactating dairy cows. *Journal of Dairy Science* 85, 1165-1175.
- McBride, B.W. & Kelly, J.M., 1990. Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. *Journal of Animal Science* 68, 2997-3010.
- Milligan, L.P. & McBride, B.W., 1985. Energy costs of ion pumping by animal tissues. *Journal of Nutrition* 115, 1374-1382.
- Mills, J.A.N., France, J. & Dijkstra, J., 1999. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 1. Dietary starch characterisation and ruminal starch digestion. *Journal of Animal Feed Science* 8, 291-340.
- Mills, J.A.N., Dijkstra, J., Bannink, A., Cammell, S.B., Kebreab, E. & France, J., 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: model development, evaluation, and application. *Journal of Animal Science* 79, 1584-1597.
- Murphy, M.R., Baldwin, R.L. & Koong, L.J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. *Journal of Animal Science* 55, 411-421.

- Mroz, Z., Koopmans, S.J., Bannink, A., Partanen, K., Krasucki, W., Øverland, M., & Radcliffe, S., 2005. Carboxylic acids as bioregulators and gut growth promoters in non-ruminants. In: R. Mosenthin & T. Zebrowska (Eds.), *Biology of the Intestine*, Elsevier, Amsterdam, The Netherlands, pp. 81-133.
- Nagorcka, B.N., Gordon, G.L.R. & Dynes, R.A., 2000. Towards a more accurate representation of fermentation in mathematical models of the rumen. In: J.P. McNamara, J. France & D.E. Beever (Eds.), *Modelling Nutrient Utilization in Farm Animals*. CAB International, Wallingford, United Kingdom, pp. 37-48.
- Nocek, J.E. & Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *Journal of Dairy Science* 74, 3598-3629.
- Oba, M. & Allen, M.S., 2003. Effect 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.
- Offner, A. & Sauvant, D., 2006. Thermodynamic modelling of ruminal fermentations. *Animal Research* 55, 1-23.
- Pellikaan, W.F., 2004. Passage of ¹³C-labelled feed components through the digestive tract of dairy cows. PhD Thesis, Wageningen University, Wageningen, The Netherlands.
- Pitt, R.E., Van Kessel, J.S., Fox, D.G., Pell, A.N., Barry, M.C. & Van Soest, P.J., 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. *Journal of Animal Science* 74, 226-244.
- Piwonka, E.J., Firkins, J.L. & Hull, B.L., 1994. Digestion in the rumen and total tract of forage-based diets with starch or dextrose supplements fed to Holstein heifers. *Journal of Dairy Science* 77, 1570-1579.
- Ramangasoavina, B. & Sauvant, D., 1993. Validation compare de 3 modèles de digestion ruminale pour prédire les flux azotés duodénaux microbiens. *Annales de Zootechnie* 42,164-165.
- Rechkemmer, G., Gabel, G., Diernæs, L., Sehested, J., Moller, P.D. & Von Engelhardt, W., 1995. Transport of short-chain fatty acids in the forestomach and hindgut. In: W. Von Engelhardt, S. Leonhard-Marek, G. Breves & D. Gieseke (Eds.), *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*. Ferdinand Enke Verlag, Stuttgart, Germany, pp. 93-113.
- Reijs, J.W., Bannink, A., Bosma, P., Lantinga, E.A. & Dijkstra, J., 2007. Modelling the effect of nutritional strategies for dairy cows on the composition of excreta N. *Submitted to Livestock Science*.
- Rémond, D., Ortigues, I. & Jouany, J.-P., 1995. Energy substrates for the rumen epithelium. *Proceedings of the Nutrition Society* 54, 95-105.
- Resende Júnior, J.C., Pereira, M.N., Boer, H. & Tamminga, S., 2006. Comparison of techniques to determine the clearance of ruminal volatile fatty acids. *Journal of Dairy Science* 89, 3096-3106.

- Reynolds, C.K. & Huntington, G.B., 1988a. Partition of portal-drained visceral net flux in beef steers. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. *British Journal of Nutrition* 60, 539-551.
- Reynolds, C.K. & Huntington, G.B., 1988b. Partition of portal-drained visceral net flux in beef steers. 2. Net flux of volatile fatty acids, D-B-hydroxybutyrate and L-lactate across stomach and post-stomach tissues. *British Journal of Nutrition* 60, 553-562.
- Reynolds, C.K., Harmon, D.L. & Cecava, M.J., 1994. Absorption and delivery of nutrients for milk protein synthesis by portal-drained viscera. *Journal of Dairy Science* 77, 2787-2808.
- Reynolds, C.K., Aikman, P., Lupoli, B., Humphries, D.J. & Beever, D.E., 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *Journal of Dairy Science* 86, 1201-1217.
- Rotger, A., Ferret, A., Calsamiglia, S. & Manteca, X., 2006. Effects of nonstructural carbohydrates and protein sources on intake, apparent total tract digestibility, and ruminal metabolism in vivo and in vitro with high-concentrate beef cattle diets. *Journal of Dairy Science* 84, 188-1196.
- Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J. & Sniffen, C.J., 1992. A Net Carbohydrate and Protein System for evaluating cattle diets. 1. Ruminal fermentation. *Journal of Animal Science* 70, 3551-3561.
- Scaife, J.R. & Tichivangana, J.Z., 1980. Short chain acyl-CoA synthetases in ovine rumen epithelium. *Biochimica et Biophysica Acta* 619, 445-450.
- Schröder, J.J., Jansen, A.G. & Hilhorst, G.J., 2005. Long term nitrogen fertilizer value of cattle slurry. *Soil Use and Management* 21, 196-204.
- Schröder, J.J., Bannink, A. & Kohn, R.A., 2006. Improving the efficiency of nutrient use on cattle operations. In: E. Pfeffer & A. Hristov (Eds.), *Nitrogen and Phosphorus Nutrition in Cattle*. CAB International, Wallingford, United Kingdom, pp. 255-279.
- Seal, C.J., Parker, D.S. & Avery, P.J., 1992. The effect of forage and forage-concentrate diets on rumen fermentation and metabolism of nutrients by the mesenteric- and portal-drained viscera in growing steers. *British Journal of Nutrition* 67, 355-370.
- Seal, C.J. & Reynolds, C.K., 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. *Nutrition Research Reviews* 6, 185-208.
- Seal, C.J. & Parker, D.S., 1994. Effect of intraruminal propionic acid infusion on metabolism of mesenteric- and portal-drained viscera in growing steers fed a forage diet: I. Volatile fatty acids, glucose, and lactate. *Journal of Animal Science* 72, 1325-1334.
- Seo, S., Tedeschi, L.O., Lanzas, C., Schwab, C.G. & Fox, D.G., 2006. Development and evaluation of empirical equations to predict feed passage rates in cattle. *Animal Feed Science and Technology* 128, 67-83.
- Shabi, Z., Bruckental, I., Zamwell, S., Tagari, H. & Arieli, A., 1999. Effects of extrusion of grain and feeding frequency on rumen fermentation, nutrient digestibility, and milk yield and composition in dairy cows. *Journal of Dairy Science* 82, 1252-1260.

- Summers, M., McBride, B.W. & Milligan, L.P., 1988. Components of basal energy expenditure. In: A. Dobson & M.J. Dobson (Eds.), *Aspects of Digestive Physiology in Ruminants*. Comstock Publishing Associates, Ithaca, United States of America, pp. 257-285.
- Sutton, J.D., Dhanoa, M.S., Morant, S.V., France, J., Napper, D.J. & Schuller, E., 2003. Rates of Production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *Journal of Dairy Science* 86, 3620-3633.
- Sveinbjörnsson, J., Huhtanen, P. & Udén, P., 2006. The Nordic Dairy Cow Model, Karoline – development of volatile fatty acids sub-model In: E. Kebreab, J. Dijkstra, A. Bannink, W.J.J. Gerrits & J. France (Eds.). *Nutrient Digestion and Utilization in Farm Animals. Modelling Approaches*. CAB International, Wallingford, United Kingdom, pp. 1-14.
- Tamminga, S., Van Straalen, W.M., Subnel, A.P.J., Meijer, R.G.M., Steg, A., Wever, C.J.G. & Blok, M.C., 1994. The Dutch protein evaluation system: the DVB/OEB-system. *Livestock Production Science* 40, 139-155.
- Tamminga, S., Brandsma, G.G., Dijkstra, J., Van Duinkerken, G., Van Vuuren, A.M. & Blok, M.C., 2007a. Protein evaluation for ruminants: the DVE/OEB 2007 system. CVB Documentation report nr. 53., Centraal Veevoeder Bureau, Lelystad, The Netherlands.
- Tamminga, S., Bannink, A., Dijkstra, J. & Zom, R., 2007b. Feeding Strategies to Reduce Methane Loss in Cattle. ASG Report 34, Animal Sciences Group, Lelystad, The Netherlands, p. 46.
- Valk, H., Kappers, I.E. & Tamminga, S., 1996. In sacco degradation characteristics of organic matter, neutral detergent fibre and crude protein of fresh grass fertilized with different amounts of nitrogen. *Animal Feed Science and Technology* 63, 63-87.
- Van Duinkerken, G., André, G., Smits, M.C.J., Monteny, G.J. & Šebek, L.B.J., 2005. Effect of rumen-degradable protein balance and forage type on bulk milk urea concentration and emission of ammonia from dairy cow houses. *Journal of Dairy Science* 88, 1099-1112.
- Van Straalen, W.M., 1995. *Modelling of Nitrogen Flow and Excretion in Dairy Cows*. PhD Thesis Wageningen Agricultural University, Wageningen, The Netherlands.
- Van Vuuren, A.M., Krol-kramer, F., Van der Lee, R.A. & Corbijn, H., 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. *Journal of Dairy Science* 75, 2215-2225.
- Van Vuuren, A.M., Van der Koelen, C.J. & Vroon-de Bruin, J., 1993. Ryegrass versus corn starch or beet pulp fibre diet effects on digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science* 76, 2692-2700.
- Williams, A.G. & Coleman, G.S., 1997. The rumen protozoa. In: P.N. Hobson & C.S. Stewart (Eds), *The Rumen Microbial Ecosystem, 2nd Edition*. Elsevier Applied Science, London, United Kingdom, pp. 73-139.

Summary & samenvatting

Summary

There is a general recognition that understanding rumen function is an important key to gain insight in the relationship between feeding and the digestion of feed, the absorption of nutrients and the synthesis of milk components. Consequently, many modelling efforts have been published to quantify aspects or even to quantify whole rumen function. With respect to the latter, elaborate models have been developed in an attempt to integrate the available knowledge on various details of the rumen fermentation processes and the underlying mechanisms involved. These models differ substantially in their conceptual approach, partly as a result of differences in the specific aim they were developed for, and in the level of detail they represent. Consequently, extant models of whole rumen function vary from empirical, static approaches following the approach adopted in current protein evaluation systems, to mechanistic, dynamic approaches that represent the impact of feeding strategy on the rumen ecosystem dynamics, based on underlying mechanisms and components. The latter category of models offers specific advantages to predict effects of feeding strategy on rumen function, digestion, nutrient absorption and nutrient utilization for milk production by the dairy cow. The same advantage becomes apparent when such models are applied to address problems related to the excretion of urine and faeces by cows and emissions to the environment.

At the start of the present study three mechanistic models were available from literature (Baldwin *et al.*, 1987; Danfær, 1990; Dijkstra *et al.*, 1992), each of which could be qualified as one of the most detailed and truly dynamic in the representation of rumen fermentation processes. Although some evaluations and model comparisons were already available from literature, they all lacked detail on scope of evaluation and insight in the underlying differences between the models in their mathematical formulation. The aim of the present study was to explore the applicability of the three models, to compare and evaluate them, to discriminate between them, and subsequently to make further improvements in the representation of whole rumen function. The study started with an analysis of the concepts adopted in the three models with particular emphasis on some model-specific parameter inputs that proved to be difficult to estimate from *in vivo* observations (Chapter 2). Apart from daily dry matter intake and diet composition as model inputs, each model required its own set of additional diet-specific parameter inputs. These inputs involved either the daily profile of acidity (pH) of rumen fluid, the parameterization of a mechanism of particle dynamics and particle comminution, or a partition factor for attributing total rate of carbohydrate fermentation to individual types of carbohydrate. It was shown that manipulating these diet-specific parameter inputs had a profound impact on simulated rumen function. For most of these inputs it was not clear, however, how they should be estimated from *in vivo* observations. Further, it was concluded that, in order to overcome this limitation, future modelling efforts should focus on the refinement of the mechanism behind these parameters.

It was concluded that the model of Baldwin *et al.* (1987) qualifies best for predicting rumen pool sizes in combination with the flows to and from these pools because of its representation of the mechanism of particle dynamics. The representation of the model of Dijkstra *et al.* (1992) relates best to available observations of rumen function and it contains the most comprehensive integration of current theories on the functioning of rumen micro-organisms.

A subsequent comparison of the mathematical formulation of the extra-microbial and the microbial processes (Chapter 3) allowed dissection of the consequences of different formulations applied in the three models. Several elements of the representation of extra-microbial processes were subsequently reformulated in order to make them identical in all models. Reformulations involved the introduction of observed fractional passage rates of fluid and particles as model inputs, equalizing non-dietary inputs to the rumen (recycling of urea and inflow of saliva), inclusion of pools of soluble protein and undegradable protein and cell wall carbohydrates, equalizing the fractional rate of absorption of volatile fatty acids (VFA) and the fractional rate of substrate degradation, equalizing molecular weights of substrates, equalizing coefficients of VFA yields from fermented substrate, and removal of a mechanism of particle dynamics. Most reformulations had a substantial impact on simulated rumen function. After these reformulations the models were identical with respect to the representation of the rumen environment of micro-organisms and of the fractional rate of substrate degradation. Remaining differences in simulation results could then be fully attributed to the formulation of the microbial processes. The results indicated that the three models simulated a fairly similar rate of substrate degradation and response to changes in dietary treatment. However, large differences remained for the partitioning of substrate incorporated into microbial matter and substrate fermented into VFA. Substantial differences also remained in the simulated flows of feed substrates and of some end-products of fermentation (VFA, ammonia and microbial mass). Compared to the outcomes of the model of Dijkstra *et al.* (1992), the total amount of carbohydrates fermented, carbohydrates incorporated in microbial mass and carbohydrates flowing out of the rumen differed up to 23, -75 and 100%, respectively, between the models. Similar large differences remained for the simulated amount of microbial protein synthesized from ammonia or soluble protein, and for the amount of feed protein fermented or flowing out of the rumen. It was concluded that models have been calibrated to deliver reasonable predictions of the more general outcomes reported in literature (organic matter, crude protein, microbial protein) but that differences in the representation of the underlying mechanisms are considerable. Further experimental identification and discrimination between the alternative representations of microbial response to substrate availability becomes important in situations where detailed aspects of microbial activity are of interest.

After delineating the underlying differences in mathematical formulation, the predictive performance of the original versions of the three models was evaluated against independent

data from our own research facilities with lactating cows fed grass based diets (Chapter 4). Outcomes of the three models were compared with identical inputs. In line with earlier findings (Chapters 2 & 3), the results of this model evaluation indicated consistent differences between the models. Again, predictions of microbial metabolism differed in particular, but differences in the prediction of individual processes compensated for each other. As a result all models predicted duodenal flow of non-ammonia-N, microbial N and organic matter reasonably well. None of the models seemed to predict all nutrient flows best and considerable differences remained in predicted flow of individual nutrients. The model of Dijkstra *et al.* (1992) seemed to give the most realistic response with respect to outflow of soluble carbohydrates and starch. Most accurate prediction of the molar proportion of individual VFA was obtained with the representation of a pH dependent VFA yield from rapidly fermentable carbohydrates that was introduced in the updated model of Baldwin *et al.* (1987).

Accurate prediction of the amount and type of VFA produced in the rumen is of major importance to progress in the prediction of cow performance from the profile of nutrients absorbed from the digestive tract. In previous efforts of rumen modelling, and in the present study (Chapters 3 & 4), this importance was recognized and the need for improvement identified. Therefore, a subsequent study was performed to investigate to what extent selected elements of rumen models are most responsible for the inaccurate prediction of VFA (Chapter 5). The model of Dijkstra *et al.* (1992) was evaluated on five aspects: the time function of feed ingestion, the distinction between substrates of roughage and concentrate origin, the kinetics of VFA absorption from the rumen, the coefficients of VFA yield from fermented substrate, and the representation of a mechanism of particle dynamics. The simulation results suggested that inaccuracy of predicted molar proportions of individual VFA is likely caused by erroneous coefficients of VFA yield or the incorrect representation of VFA absorption kinetics. The other three aspects appeared of less importance to explain errors in predicted VFA molar proportions.

With the aim to improve the representation of VFA yield from fermented substrate in the rumen of lactating cows, a new set of VFA coefficients was derived (Chapter 6). A regression model was formulated that describes the conversion of substrate (soluble carbohydrates, starch, hemi-cellulose, cellulose, protein) into individual VFA (acetate, propionate, butyrate, other VFA). Inputs to the model were rates of substrate digestion in the rumen fluid, and outputs were calculated molar proportions of VFA produced from digested substrate. The estimation accuracy of the regression model was tested by performing some simulation studies. Data sets were generated from a known set of stoichiometric coefficients of VFA yield with random and normally distributed error added with increasing variation to calculated VFA production rates. Regression of the model against these simulated data sets indicated that with increasing variation of the random error added, a lower fraction of the variation in VFA molar proportion could be explained by the model. However, estimates of

VFA coefficients remained accurate, even with the highest variation. This result demonstrated that the method has a good potential to deliver reasonable estimates of VFA coefficients based on regressions against *in vivo* data. *In vivo* data were obtained from observations in high-yielding lactating cows of Friesian breeds (mostly Holstein-Friesian) performed in 47 rumen digestion trials with 182 dietary treatments tested. Coefficient estimates were obtained which differ from those previously published. A separate set of coefficients for roughage-rich and concentrate-rich diets still appeared necessary because of significant differences in the coefficient estimates for acetate and propionate yield for these two types of diet. Only a small fraction of the observed variation in molar proportion of individual VFA could be explained, but results closely resembled those obtained with the simulated data sets. The new coefficient estimates improve the prediction of VFA yield in the rumen of lactating cows because observations were used from exclusively lactating cows. When applying these coefficient estimates to rumen models, possible differences between assumptions and concepts used in the regression model and the actual rumen model have to be included in the analysis.

The regression results also indicated that coefficient values were estimated with less accuracy from the data set of concentrate-rich diets in comparison to that of roughage-rich diets. Combined with the fact that a separate set of coefficients was needed for roughage-rich and concentrate-rich diets, this led to further investigation of the background of this variation. Based on earlier findings in the present study (Chapters 4 & 5) and indications from literature, the regression model was extended with a representation of VFA absorption kinetics and VFA outflow with rumen fluid (Chapter 7). Furthermore, the coefficients of VFA yield from soluble carbohydrates and starch were made dependent on pH of rumen fluid. According to the regression results, acetate formation from soluble carbohydrates decreases with a decrease of pH, whereas propionate formation from soluble carbohydrate and starch increases. Butyrate formation from soluble carbohydrate increases but that from starch decreases upon a reduced pH. Despite the additional details included in the regression model, there was still a need for separate coefficient estimates for roughage-rich and concentrate-rich diets. It was concluded that the effect of rumen fermentation conditions on the type of VFA produced has to be included in modelling efforts that aim to predict VFA supply to the cow.

Predicting the VFA supply to the cow required consideration of some further aspects. Firstly, the dynamics of VFA transport to blood by the rumen wall affects the VFA molar proportions in rumen fluid, the intra-luminal VFA concentrations to which micro-organisms and the rumen wall are exposed, and the contribution of transport by the rumen wall and outflow with rumen fluid to VFA clearance. Secondly, VFA are the main source of energy used by rumen epithelia. Investigation of its importance required intra-epithelial VFA metabolism to be integrated with the dynamics of VFA transport and the mechanisms of adaptation of these epithelial tissues. In contrast to the empirical treatment (or no representation) of these aspects in previous modelling efforts, a mechanistic model was

constructed (Chapter 7) in which both aspects were represented in an integrated manner. The model allows the evaluation of the interactions between feeding strategy, rumen conditions (VFA production rates, VFA concentrations in rumen fluid, fluid outflow), functionality of the rumen wall (shape of rumen papillae, epithelial mass, transport capacity of epithelia, metabolic activity of epithelial tissue wall) and appearance of rumen VFA in portal blood. Adaptation of the rumen wall was represented according to recent experimental findings at our own research facilities. Representation of VFA transport was based on experimental findings reported in literature. The representation of intra-epithelial metabolism of VFA was based on enzyme assays of CoA-synthetase activity. Some essential characteristics of VFA transport and intra-epithelial metabolism could be reproduced by the model. Simulation results demonstrated the impact of VFA production rate, VFA molar proportions and epithelial tissue mass on the fraction of VFA metabolised or appearing in portal blood. Simulation results clearly indicated variation in the fraction metabolised or absorbed. It was concluded that representation of the interactions between intra-ruminal VFA formation, VFA transport across the rumen wall, intra-epithelial VFA metabolism and adaptation of rumen epithelia broadens the range of questions that may be answered by rumen models. It is also emphasized, however, that the model needs extension and further development. The model needs to be elaborated by linkage of VFA metabolism to energy requirements for distinct physiological functions (cell maintenance, tissue proliferation, protein turnover, transport of nutrients and ions). There is a definite need for more experimental data on, firstly, the activity of CoA-synthetase and the competitive inhibition between VFA, and secondly, on the adaptive response of epithelial tissues and functionality of the rumen wall. Experimental data should more match the purpose for which they are obtained, which in this respect means they need to be obtained from lactating cows as the target animal.

The General Discussion (Chapter 8) outlines the most important elements that require representation in models of rumen function. The improved representation of VFA yield from fermented substrate was evaluated against other approaches reported in literature. Also the various levels of aggregation that may be included were discussed. A first level has now been widely adopted and involves the consideration of intrinsic degradation characteristics, passage rate of soluble and particulate matter and yield of end-products of fermentation. A next level is reached with the concept that environmental conditions have an important modifying effect on microbial activity and the dynamics of rumen fermentation. A following level is reached with the representation of the impact of rumen wall functionality on these environmental conditions and representation of its adaptation to various feeding strategies. Finally, a discussion is included on future modelling efforts, on the range of research topics in which these models may be applied, and on their potential to accommodate for new findings from fundamental research and to develop nutrient based feed evaluation systems.

Main conclusions from this thesis

- A direct comparison and evaluation of three extant mechanistic, dynamic models of rumen function indicated that the models of Dijkstra *et al.* (1992) and Baldwin *et al.* (1987) performed best in predicting rumen outflow of organic matter and non-ammonia N. The model of Dijkstra *et al.* (1992) performed best in predicting starch outflow. Otherwise evaluation results remained inconclusive.
- The models strongly differ in their diet-specific parameter inputs. Some inputs are difficult to estimate because their concept does not match available observations. In this case, models need further development, or they need to make use of inputs more readily available as with the model of Dijkstra *et al.* (1992).
- Despite the apparent similarity of mechanistic, dynamic models of rumen function in their performance to predict nutrient digestion, major differences remain in the representation of the underlying mechanism. Such differences become important when studying details of the microbial response to substrate availability.
- An improved representation of VFA yield was obtained by regressions against *in vivo* observations of rumen digestion in the target animal, and by including the effect of rumen fluid pH on VFA yield from rapidly fermentable carbohydrates.
- Functionality of the rumen wall (morphology, epithelial mass, transport capacity, intra-epithelial VFA metabolism) is an important determinant of the dynamics of rumen VFA and deserves more attention when modelling whole rumen function.

Samenvatting

Het wordt algemeen onderkend dat een goed begrip van de penswerking een belangrijke sleutel is voor het verkrijgen van inzicht in de relatie tussen voerstrategie en de vertering van het voer, de absorptie van nutriënten en de synthese van melkcomponenten. Uitgaande van het belang van de pens, zijn er verschillende modelleerstudies gepubliceerd om aspecten van penswerking, of zelfs de gehele penswerking te kwantificeren. Met betrekking tot dit laatste zijn er meer uitgebreide modellen ontwikkeld als poging om tot een integratie te komen van de beschikbare kennis rondom uiteenlopende details van de fermentatieprocessen in de pens en van hun onderliggende mechanismen. Deze modellen verschillen substantieel in hun conceptuele benadering, wat gedeeltelijk het gevolg is van de verschillen in het doel waarvoor ze ontwikkeld werden en in het niveau van detail dat werd weergegeven. Om die reden variëren bestaande modellen voor de penswerking van een empirische, statische benadering die lijkt op de benadering in de huidige eiwitwaarderingssystemen, tot mechanistische, dynamische benaderingen die gericht zijn op het weergeven van de invloed van de gevolgde voerstrategie op de dynamiek van het pensecosysteem, gebaseerd op de onderliggende mechanismen en componenten. Deze laatste categorie modellen biedt specifieke voordelen met betrekking tot het voorspellen van effecten van voerstrategie op het functioneren van de pens, de vertering, de absorptie van nutriënten en de nutriëntenbenutting voor melkproductie door de koe. Dezelfde voordelen komen naar voren indien toepassing van deze modellen zich richt op problemen die gerelateerd zijn aan de excretie van urine en feces door koeien en de emissies naar het milieu.

Aan het begin van de huidige studie waren er in de literatuur drie mechanistische modellen beschikbaar (Baldwin *et al.*, 1987; Danfær, 1990; Dijkstra *et al.*, 1992), die elk gekwalificeerd konden worden als sterk gedetailleerd en met een werkelijk dynamische benadering bij het weergeven van de fermentatieprocessen in de pens. Reeds enkele modevaluaties en modelvergelijkingstudies waren verschenen in de literatuur, echter er was vanuit het oogpunt van modevaluatie in alle gevallen sprake van een gebrek aan detail en inzicht in de onderliggende verschillen in mathematische formulering in deze modellen. Het doel van de huidige studie was het verkennen van de toepasbaarheid van deze drie modellen, het vergelijken en evalueren van hen, het discrimineren tussen hen, en vervolgens het aanbrenge van verbeteringen in de weergave van penswerking. De studie startte met een analyse van de concepten die zijn toegepast in deze drie modellen met speciale aandacht voor enkele rantsoenspecifieke invoerparameters waarvan gebleken was dat deze lastig zijn te schatten uit *in vivo* waarnemingen (Hoofdstuk 2). Naast de dagelijkse voeropname en de rantsoensamenstelling, vroeg ieder model om haar eigen set van aanvullende rantsoenspecifieke invoerparameters. Het betrof ofwel het dagelijkse profiel van de zuurgraad (pH) van pensvloeistof, ofwel de parameterisering van een mechanisme van deeltjeskinetiek en deeltjesverkleining, ofwel passagesnelheden en een verdelingsfactor voor het verdelen van

de totale koolhydraatfermentatie over afzonderlijke typen koolhydraten. Aangetoond werd dat het aanpassen van de waarde van deze invoerparameters een sterke invloed heeft op de gesimuleerde penswerking. Voor de meeste van deze invoerparameters werd echter niet duidelijk hoe deze geschat kunnen worden uit *in vivo* waarnemingen. Geconcludeerd werd dat deze beperking kan worden ondervangen door in toekomstige modelleerstudies aandacht te besteden aan de verfijning van de weergave van de mechanismen die bij deze parameters betrokken zijn. Verder werd geconcludeerd dat het model van Baldwin *et al.* (1987) zich het best kwalificeert voor wat betreft de voorspelling van zowel de hoeveelheid materiaal in de pens als ook de in- en uitstroom van dit materiaal vanwege de weergave van een mechanisme van deeltjeskinetiek. De weergave in het model van Dijkstra *et al.* (1992) komt het best overeen met beschikbare waarnemingen van de penswerking en het bevat de meest uitgebreide integratie van huidige theorieën op het gebied van het functioneren van micro-organismen in de pens.

In een vervolgstudie werd de mathematische formulering van de extramicrobiële en de microbiële processen vergeleken (Hoofdstuk 3) die het mogelijk maakte om te ontleden wat de gevolgen zijn van de verschillende formuleringen in deze drie modellen. Verschillende onderdelen in de weergave van de extramicrobiële processen werden achtereenvolgens geherformuleerd om ze identiek te maken in de drie modellen. De herformuleringen betroffen het introduceren van waargenomen fractionele passagesnelheden van vloeistof en deeltjes als invoerparameters, het gelijkmaken van de instroom van niet-rantsoen bestanddelen in de pens (recyclen van ureum en speekselinstroom), het invoegen van oplosbaar eiwit en niet-afbreekbaar eiwit en celwandkoolhydraten als modelvariabelen, het gelijkmaken van de fractionele snelheid van de absorptie van vluchtige vetzuren (VVZ) en afbraak van afbreekbaar substraat, het gelijkmaken van molecuulgewichten van substraat, het gelijkmaken van de coëfficiënten voor de vorming van VVZ uit gefermenteerd substraat, en het verwijderen van het mechanisme van deeltjeskinetiek. De meeste formuleringen hadden een substantiële invloed op het gesimuleerde functioneren van de pens. Na alle herformuleringen waren de modellen identiek voor wat betreft de weergave van het milieu van micro-organismen en van de fractionele afbraaksnelheid van afbreekbaar substraat. De resterende verschillen in simulatieresultaten konden dan volledig worden toegeschreven aan de formulering van de microbiële processen. Uit de resultaten bleek dat de modellen een redelijk vergelijkbare substraatafbraak en respons op veranderingen in rantsoen simuleerden. Grote verschillen bleven echter aanwezig in de gesimuleerde verdeling van substraatverbruik tussen inbouw in microbiële massa en omzetting naar VVZ. Ook bleven grote verschillen aanwezig in de gesimuleerde uitstroom van voersubstraten en van sommige fermentatie-eindproducten (VVZ, ammoniak en microbiële massa). Ten opzichte van de uitkomsten met het model van Dijkstra *et al.* (1992) verschilde de totale hoeveelheid gefermenteerde koolhydraten, koolhydraten ingebouwd in microbiële massa en koolhydraten die uit de pens stromen respectievelijk 23, -75 en 100% tussen de modellen. Vergelijkbaar grote verschillen bleven

aanwezig in de gesimuleerde hoeveelheid microbiële eiwit die gevormd werd uit ammoniak of oplosbaar eiwit als N-bron, en in de hoeveelheid voereiwit die uit de pens stroomt. Geconcludeerd werd dat de modellen gekalibreerd zijn om redelijke voorspellingen te geven van de meer algemene uitkomsten die gerapporteerd worden in de literatuur (vertering van organische stof, ruw eiwit en zetmeel) maar dat verschillen in de weergave van de onderliggende mechanismen aanzienlijk zijn. Aanvullende experimentele identificatie en discriminatie tussen deze alternatieve weergaven van de microbiële respons op substraataanbod wordt belangrijk in situaties waarbij er interesse is in de details rondom microbiële activiteit.

Na het ontrafelen van de onderliggende verschillen in wiskundige formulering, werd de voorspellingskracht van de originele versie van de drie modellen geëvalueerd met onafhankelijke experimentele gegevens uit onze eigen onderzoeksfaciliteiten met lacterende melkkoeien die op gras gebaseerde rantsoenen gevoerd kregen (Hoofdstuk 4). Uitkomsten van de drie modellen werden vergeleken bij dezelfde modelinvoer. In overeenstemming met de eerdere bevindingen (Hoofdstukken 2 & 3), gaven ook de uitkomsten van deze model-evaluatie aan dat er consistente verschillen bestaan tussen de modellen. Opnieuw verschilden vooral de voorspellingen van het microbiële metabolisme, maar de verschillen in de voorspelling van afzonderlijke processen compenseerden elkaar. Als gevolg hiervan gaven alle modellen een redelijke voorspelling van de uitstroom van niet-ammoniak-N, microbiële N en organische stof naar de dunne darm. Geen van de modellen leek alle nutriëntenstromen het best te voorspellen en aanzienlijke verschillen bleven aanwezig in de voorspelling van individuele nutriëntenstromen. Het model van Dijkstra *et al.* (1992) gaf de meest realistische respons met betrekking tot de uitstroom van oplosbare koolhydraten en zetmeel. De meest nauwkeurige voorspelling van de molaire verhouding van individuele VVZ werd verkregen met de weergave van een pH-afhankelijke VVZ-vorming uit snelfermenteerbare koolhydraten die was geïntroduceerd in de aangepaste versie van het model van Baldwin *et al.* (1987).

Nauwkeurige voorspelling van de hoeveelheid en het type VVZ dat gevormd wordt in de pens is van groot belang om vooruitgang te boeken bij het voorspellen van de respons van een melkkoe op het profiel aan nutriënten dat geabsorbeerd wordt vanuit het maagdarmkanaal. In eerdere modelleerstudies, en in de huidige studie (Hoofdstukken 3 & 4), werd dit belang onderkend en de noodzaak voor verbetering benadrukt. Om die reden werd een vervolgstudie verricht om na te gaan in welke mate enkele geselecteerde aspecten van de modellen verantwoordelijk zijn voor de onnauwkeurige VVZ voorspellingen (Hoofdstuk 5). Het model van Dijkstra *et al.* (1992) werd geëvalueerd op vijf aspecten: de tijdsfunctie voor de opname van voer, het onderscheid tussen substraat uit ruwvoer- en krachtvoerbronnen, de kinetiek van VVZ-absorptie door de penswand, de coëfficiënten voor VVZ-vorming uit gefermenteerd substraat, en de weergave van een mechanisme voor deeltjeskinetiek. De simulatie-uitkomsten suggereerden dat een onnauwkeurig voorspelde molaire verhouding van

VVZ waarschijnlijk veroorzaakt wordt door onnauwkeurige coëfficiënten voor VVZ-vorming of een foutieve weergave van de kinetiek van VVZ-absorptie. De overige drie aspecten bleken van ondergeschikt belang bij het verklaren van fouten in de voorspelde molaire verhouding van VVZ.

Met als doel de weergave van VVZ-vorming uit gefermenteerd substraat in de pens van melkvee te verbeteren, werd een nieuwe set VVZ-coëfficiënten afgeleid (Hoofdstuk 6). Een regressiemodel werd geformuleerd dat de omvorming van substraat (oplosbare koolhydraten, zetmeel, hemi-cellulose, cellulose, eiwit) in de afzonderlijke VVZ (azijnzuur, propionzuur, boterzuur, overige VVZ) beschrijft. Invoer voor het model waren waargenomen verteringssnelheden van substraat in de pens, en uitvoer was de berekende molaire verhouding waarmee VVZ gevormd worden uit de afzonderlijke substraten. De nauwkeurigheid van de schattingsmethode werd getest door enkele simulatiestudies uit te voeren. Datasets werden gegenereerd vanuit een bekende set stoichiometrische coëfficiënten voor de vorming van VVZ, onder toevoeging van een aselechte en normaal verdeelde fout met toenemende variatie aan de berekende VVZ-productiesnelheden. Regressie van het model tegen deze gesimuleerde data sets duidde aan dat met toenemende variatie van de aselechte fout die werd toegevoegd, een kleinere fractie van de molaire VVZ-verhouding verklaard kon worden door het model. De schattingen van de VVZ-coëfficiënten bleven echter nauwkeurig, ook bij hoogste variatie. Deze uitkomst gaf aan dat de methode potentie heeft om redelijke schattingen te verkrijgen van *in vivo* VVZ-coëfficiënten gebaseerd op regressies tegen *in vivo* gegevens. De *in vivo* gegevens werden ontleend aan waarnemingen in hoogproductief melkvee van Friesian rassen (meestal Holstein-Friesian) uitgevoerd in 47 pensverteringsstudies waarin 182 nutritionele behandelingen werden getest. De geschatte coëfficiëntwaarden waren verschillend van eerder gepubliceerde waarden. Een afzonderlijke set coëfficiënten voor ruwvoerrijke en voor krachtvoerrijke rantsoenen bleek nodig vanwege significante verschillen tussen de geschatte coëfficiëntwaarden voor azijnzuur- en propionzuurproductie voor deze beide rantsoenklassen. Slechts een klein deel van de waargenomen variatie in het molaire aandeel van individuele VVZ kon worden verklaard, maar de regressieresultaten leken sterk op die verkregen met de gesimuleerde datasets. De nieuwe coëfficiëntwaarden worden geacht de voorspelling van de VVZ-vorming in de pens te verbeteren omdat ze ontleend zijn aan alleen maar waarnemingen bij lacterende melkkoeien. Bij het toepassen van deze coëfficiëntwaarden in modellen die de pensfunctie beschrijven, moeten mogelijke verschillen in aannames en concepten tussen het regressiemodel en het betreffende model ter beschrijving van de pensfunctie in de studie betrokken worden.

Uit de regressieresultaten kwam ook naar voren dat de VVZ-coëfficiënten met een lagere nauwkeurigheid konden worden geschat uit de dataset voor krachtvoerrijke rantsoenen dan uit die voor ruwvoerrijke rantsoenen. In combinatie met het feit dat een afzonderlijke set VVZ-coëfficiënten nodig bleek voor ruwvoerrijke en voor krachtvoerrijke rantsoenen, leidde dit tot verder onderzoek naar de achtergronden van deze variatie. Gebaseerd op eerdere

uitkomsten (Hoofdstukken 4 & 5) en indicaties uit de literatuur werd het regressiemodel uitgebreid met een weergave van de kinetiek van VVZ-absorptie en VVZ-uitstroom met pensvloeistof (Hoofdstuk 7). Bovendien werden de coëfficiënten voor de vorming van VVZ uit gefermenteerde oplosbare koolhydraten en zetmeel afhankelijk gemaakt van de pH van pensvloeistof. De regressieresultaten gaven aan dat met dalende pH de azijnzuurvorming uit oplosbare koolhydraten afneemt, terwijl de vorming van propionzuur uit oplosbare koolhydraten en zetmeel dan juist toeneemt. De vorming van boterzuur uit oplosbare koolhydraten neemt toe terwijl die uit zetmeel afneemt bij afnemende pH. Ondanks de toevoeging van aanvullende details in het regressiemodel was het nog steeds noodzakelijk om aparte sets VVZ-coëfficiënten af te leiden voor ruwvoerrijke en krachtvoerrijke rantsoenen. Geconcludeerd werd dat het effect van de fermentatieomstandigheden in de pens op het type VVZ dat gevormd wordt dient te worden meegenomen in modelleerstudies die gericht zijn op het voorspellen van het VVZ-aanbod aan de melkkoe.

Voor een voorspelling van het VVZ-aanbod aan de melkkoe dienen enkele aanvullende aspecten in ogenschouw te worden genomen. Ten eerste, de dynamiek van het transport van VVZ naar het bloed door de penswand beïnvloedt de molaire VVZ-verhouding in de pens, de intra-luminale VVZ-concentraties waaraan de micro-organismen en de penswand worden blootgesteld, en de bijdrage van transport door de penswand en uitstroom met pensvloeistof aan de verdwijning van VVZ uit de pens. Ten tweede, VVZ zijn de belangrijkste energiebron voor de epitheelweefsels in de penswand. Het onderzoeken van het belang van dit metabolisme vraagt om een integratie van intra-epitheliaal metabolisme met de dynamiek van VVZ transport en de aanpassingsmechanismen in deze epitheelweefsels. In tegenstelling tot een empirische benadering (of geen enkele weergave) van deze aspecten in eerdere modelleerstudies, werd in deze studie een mechanistisch model ontwikkeld (Hoofdstuk 7) waarin beide aspecten op geïntegreerde wijze zijn weergegeven. Het model maakt het mogelijk om de interactie te verkennen tussen voerstrategie, fermentatieomstandigheden in de pens (VVZ- productiesnelheden, VVZ-concentraties, pH, fractionele uitstroomsnelheid pensvloeistof), de functionaliteit van de penswand (vorm van penspapillen, epitheelmassa, transportcapaciteit in epitheel, metabole activiteit van epitheelweefsel) en de verschijning van in de pens geproduceerde VVZ in portaal bloed. Aanpassing van de penswand werd weergegeven op basis van recente experimentele uitkomsten in onze onderzoeksfaciliteiten. De weergave van VVZ-transport werd gebaseerd op in de literatuur gerapporteerde experimentele uitkomsten. De weergave van het intra-epitheliale metabolisme van VVZ werd gebaseerd op uitkomsten van enzym assays voor de activiteit van CoA-synthetases. Het model bleek enkele kenmerkende eigenschappen van het transport en intra-epitheliale metabolisme van VVZ te kunnen reproduceren. Simulatieresultaten maakten duidelijk wat de invloed kan zijn van VVZ-productiesnelheden, molaire VVZ-verhouding en de epitheelmassa op de fractie VVZ die gemetaboliseerd wordt of in portaal bloed verschijnt. De simulaties

gaven duidelijk aan dat de fractie die gemetaboliseerd wordt, of geabsorbeerd, varieert. Geconcludeerd werd dat een weergave van de interactie tussen intra-luminale VVZ-productie, VVZ-transport over de penswand, intra-epitheliaal VVZ-metabolisme en aanpassing van epitheelweefsel in de penswand het bereik aan vragen waarvoor een pensmodel antwoorden kan genereren uitbreidt. Het werd echter eveneens duidelijk dat het model verdere ontwikkeling en uitbreiding behoeft. Het model dient te worden uitgebreid door het koppelen van VVZ-metabolisme met de energiebehoefte voor specifieke fysiologische functies (onderhoudsprocessen in de cel, proliferatie van epitheelweefsel, de omzetting van epitheelwit en transport van nutriënten en ionen). Er is onmiskenbaar behoefte aan meer experimentele gegevens voor, ten eerste, de activiteit van CoA-synthetases en de competitieve inhibitie tussen VVZ, en ten tweede voor de adaptatierespons van epitheelweefsel en de functionaliteit van de penswand. Er zijn experimentele gegevens nodig die beter voldoen aan het doel waarvoor ze worden verzameld, wat in dit geval inhoudt dat ze verzameld moeten worden in de lacterende melkkoe als doeldier.

De algemene discussie (Hoofdstuk 8) geeft een overzicht van de belangrijkste aspecten die weergegeven moeten worden in modellen die de functie van de pens voorspellen. De verbeterde weergave van de VVZ-vorming uit gefermenteerd substraat werd vergeleken met andere benaderingen die in de literatuur zijn gerapporteerd. Ook de verschillende aggregatieniveaus die het model kan beslaan werden bediscussieerd. Een eerste niveau wordt alom toegepast en betreft het beschouwen van de intrinsieke afbraakkenmerken, de passagesnelheden van opgelost materiaal en deeltjesmateriaal, en de opbrengst aan fermentatie-eindproducten. Een volgend niveau wordt bereikt met de notie dat de omstandigheden in de pens een belangrijk modificerend effect hebben op de microbiële activiteit en de kinetiek van pensfermentatie. Een daarop volgend niveau wordt bereikt met de weergave van de invloed van de functionaliteit van de penswand op de omstandigheden in de pens en de weergave van de aanpassing hiervan aan verschillende voedingsstrategieën. Ten slotte werd bediscussieerd welke modelleerstudies kunnen volgen in de toekomst, op welke onderzoeksterreinen deze modellen kunnen worden ingezet, en wat de potentie is van deze modellen om nieuwe uitkomsten uit fundamenteel onderzoek mee te nemen en om nieuwe op nutriënten gebaseerde voederwaardersystemen te ontwikkelen.

De belangrijkste conclusies uit dit proefschrift:

- Een directe vergelijking en een evaluatie van drie bestaande mechanistische, dynamische modellen voor de functie van de pens gaf aan dat de modellen van Dijkstra *et al.* (1992) en Baldwin *et al.* (1987) het best presteren voor wat betreft de voorspelde uitstroom van organische stof en niet-ammoniak N uit de pens. Het model van Dijkstra *et al.* (1992) lijkt de beste voorspelling te geven van de uitstroom van zetmeel. Voor het overige bleek uit de uitkomsten van de evaluatie geen duidelijke voorkeur.

- Mechanistische, dynamische modellen voor het functioneren van de pens verschillen sterk wat betreft hun rantsoen-specifieke invoerparameters. Sommige invoerparameters zijn moeilijk te schatten omdat ze conceptueel niet overeenstemmen met beschikbare experimentele waarnemingen. In dit geval behoeven deze modellen verdere ontwikkeling, of ze moeten gebruik gaan maken van meer beschikbare invoergegevens zoals het model van Dijkstra *et al.* (1992) dat doet.
- Ondanks de ogenschijnlijke overeenkomst tussen de modellen wat betreft hun vermogen om pensvertering te voorspellen, resteren er aanzienlijke verschillen in de weergave van de onderliggende mechanismen. Deze verschillen worden belangrijk als details bestudeerd worden rondom de respons van micro-organismen op substraatbeschikbaarheid in de pens.
- Een verbeterde weergave van de VVZ-vorming werd verkregen door regressies tegen *in vivo* waarnemingen van pensvertering in uitsluitend de doeldieren, en door het invoegen van het effect van de pH van pensvloeistof op de VVZ-vorming uit snel-fermenteerbare koolhydraten.
- De functionaliteit van de penswand (morfologie, epitheelmassa, transport capaciteit, intra-epitheliale metabolisme) is een belangrijke bepalende factor voor de dynamiek van VVZ in de pens en verdient meer aandacht bij het modelleren van het functioneren van de pens.

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André

Curriculum Vitae

André Bannink werd geboren op 23 mei 1962 in Ruurlo. Hij groeide op in Ruurlo, Vorden (gemeenschap 't Medler) en vervolgens opnieuw Ruurlo in de periode van 1966 tot en met 1974. Na het behalen van het Atheneum-B aan het Baudartius College in Zutphen in 1981 begon hij met de studie Biologie aan de Rijksuniversiteit Groningen. In 1988 studeerde hij af met afstudeerrichtingen Biologische Psychiatrie (*in vivo* binding aan dopamine-receptoren in hersenen) en Theoretische Biologie (weergave biologische regulatieprocessen m.b.v. negatieve feedback besturingsmechanisme). Na het afronden van zijn studie vervulde hij vanaf 1989 zijn vervangende dienstplicht bij de sectie Medische Electrotechniek van de toenmalige Hogeschool Enschede, en trad vervolgens in 1991 in tijdelijke dienst als docent. In 1992 trad hij in dienst als onderzoeker bij het toenmalige DLO-Instituut voor Veevoedingsonderzoek (IVVO) in Lelystad met als voornaamste taak het ontwikkelen van simulatiemodellen, wat tevens de basis is geweest voor het onderzoek beschreven in dit proefschrift. Het promotieonderzoek startte in 1994 onder begeleiding van prof. S. Tamminga en in samenwerking met prof. J. France en dr. J. Dijkstra. Hiervoor verbleef hij in 1995 voor langere tijd op het North Wyke Research Station van het Institute of Grassland and Environmental Research in Devon (UK). Tijdens dit verblijf kreeg het eerste deel van dit proefschrift vorm. Sinds 1992 tot op heden is hij als onderzoeker werkzaam bij het onderzoeksinstituut in Lelystad onder de naam van achtereenvolgens, het DLO-Instituut voor Veevoedingsonderzoek (IVVO), het Instituut voor Dierhouderij en Diergezondheid (ID-DLO) en momenteel de Animal Sciences Group van Wageningen Universiteit en Researchcentrum (WUR-ASG).

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