MATHEMATICAL MODELLING AND INTEGRATION OF RUMEN FERMENTATION PROCESSES

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MATHEMATICAL MODELLING AND INTEGRATION OF RUMEN FERMENTATION PROCESSES

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Proefschrift

ter verkrijging van de graad van
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dr. H.C. van der Plas,
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STELLINGEN

1. Voor een goede voorspelling van nutriënten, beschikbaar voor absorptie uit de pens van koeien gevoerd met uiteenlopende rantsoenen, is het van cruciaal belang meerdere pools van micro-organismen te onderscheiden.
   Dit proefschrift

2. De voorspelling van het type vluchtig vetzuur dat in de pens geproduceerd wordt, vereist een gedetailleerder weergave van de relevante processen dan in de huidige modellen van de pensfermentatie gebruikelijk is.
   Dit proefschrift

3. De verhoudingen waarin vluchtige vetzuren in de pens aanwezig zijn, geven zelden de verhoudingen weer waarin ze zijn geproduceerd.
   Dit proefschrift

4. Een kwantitatief begrip van de respons van protozoën op rantsoenwijzigingen en van hun effekten op de voor absorptie beschikbare nutriënten vereist een wiskundige weergave van het metabolisme van bacteriën en protozoën in de pens.
   Dit proefschrift

5. De stelling van Czerkawski (1987) dat de hoeveelheid protozoën die de pens verlaat aanzienlijk groter is dan de hoeveelheid die de lebmaag bereikt, berust op een foutieve aanname.
   Czerkawski, J.W. (1987). Reassessment of the contribution of protozoa to the microbial protein supply to the host ruminant animal. *Journal of Theoretical Biology* **126**, 335-341.
   Abe, M. & Iriki, T. (1989). Mechanism whereby holotrich ciliates are retained in the reticulo-rumen of cattle. *British Journal of Nutrition* **62**, 579-587.

6. De opmerking van Broderick *et al.* (1991), dat de consequent lagere ammoniak concentraties in de pensvloeistof van gedefauneerde herkauwers het beste bewijs is voor de stelling dat protozoën aminozuren aktief deamineren, gaat voorbij aan het geïntegreerde, dynamische karakter van de pensfermentatie.
   Broderick, G.A., Wallace, R.J. & Ørskov, E.R. (1991). Control of rate and extent of protein degradation. In *Physiological Aspects of Digestion and Metabolism in Ruminants*, pp. 541-592 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. San Diego: Academic Press Inc.

7. Wiskundige modellen zijn bij uitstek geschikt om een integratie van kennis uit diverse, uiteenlopende disciplines mogelijk te maken.

8. Ten opzichte van andere methoden is de meerwaarde van de allometrische benadering in de beschrijving van groei van zowel planten als dieren gering.
   Thornley, J.H.M. & Johnson, I.R. (1990). *Plant and Crop Modelling*. Oxford: Clarendon Press.

9. Het bedenken van een passende naam voor een te ontwikkelen wiskundig model is een bijzonder zinvol aspekt in de ontwerpfase van het model.

10. De nadruk die gelegd wordt op behoeften van dieren om een bepaald gewenst produktiedoel te bereiken, beperkt de mogelijkheden van een efficiënte, op duurzaamheid gerichte bedrijfsvoering.

11. De weerstand tegen veranderingen in de kerk is slechts dan gerechtvaardigd als deze gebaseerd is op de Bijbel als belangrijkste toetsingsmogelijkheid voor het kerk-zijn, maar vindt zijn oorsprong vaak in de angst om zekerheden in de kerk te verliezen.

12. Het aantal enkelblessures, opgelopen tijdens het beoefenen van volleybal, zou aanzienlijk gereduceerd kunnen worden indien NeVoBo regel 16.2.2.a geschrapt werd.
   NeVoBo regel 16.2.2.a: Het aanraken van het veld van de tegenpartij met één of beide voet(en) is toegestaan, mits hierbij een deel van deze voet(en) in kontakt met of boven de middenlijn blijft.

13. De in het algemeen grote hoeveelheden alkohol, die tijdens het klaverjassen genuttigd worden, doen niet vermoeden dat de deelnemers hun maat kennen.

14. Het heilig verklaren van koeien heeft negatieve gevolgen voor het milieu.

15. Als het de vakgroepen, die deelnemen aan het op te richten onderzoekinstituut Animal Sciences, lukt hun eigen identiteit en zeggenschap te bewaren en tegelijkertijd het onderzoekinstituut te doen slagen, kalft de os.

16. Het meest duurzame aspekt van duurzame produktiesystemen is tot nu toe de diskussie over de definitie van duurzaamheid.

Jan Dijkstra
Mathematical modelling and integration of rumen fermentation processes
Wageningen, 7 juni 1993

Want zie, uitspraken kunt u toetsen,
zoals u eten keurt door goed te proeven.
Elihu (Job 34:4)

VOORWOORD

Het onderzoek beschreven in dit proefschrift is tot stand gekomen bij de vakgroep Veevoeding van de Landbouwuniversiteit Wageningen. Ik ben de vakgroep zeer erkentelijk voor de mij geboden mogelijkheid om het onderzoek grotendeels naar eigen inzicht in te vullen. De uitstekende werkomstandigheden op Veevoeding zijn wijd en zijd bekend en ik wil mijn (ex-)collega's bedanken voor de zeer plezierige en stimulerende sfeer waar ik de afgelopen 5 jaar van mocht genieten. Zonder iemand te kort te willen doen, bedank ik met name Carolien Makkink en Karel de Greef (mijn keldergenoten, die er voor zorgden dat het in de AIO-kelder prima toeven was), Marianne van 't End en Tamme Zandstra (mijn paranimfen, voor de gezelligheid bij de uitstapjes, volleybal, 'Onder de Linden' etc.) en René Kwakkel (voor het luisterend oor bij allerlei probleempjes).

Voordat ik dit onderzoek in mei 1988 begon, had ik zo mijn twijfels om als 'rechtgeaard' veefokker de onbekende wereld van de veevoeding in te stappen. Kor Oldenbroek, die mij tijdens mijn fokkerijonderzoek op het Instituut voor Veeteeltkundig Onderzoek (IVO) in Zeist begeleidde, heeft me gestimuleerd om toch naar de AIO-functie bij Veevoeding te solliciteren. Kor, ik ben je bijzonder dankbaar daarvoor; de overstap is me zeer goed bevallen. Bovendien wil ik jou, Jaap de Rooy en Harrie Laurijsen bedanken voor de onderzoeksbasis die dankzij jullie tijdens mijn stage-, afstudeervak- en werkperiode op het IVO (1985-1988) is gelegd.

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Dijkstra, J., 1993. Mathematical modelling and integration of rumen fermentation processes. In ruminants, the profile of nutrients available for absorption generally differs considerably from that ingested. These differences result from the metabolic activities of the rumen microorganisms. The main aim of the present study, was to model the rumen fermentation processes, to achieve the integration and quantification of these processes, and with the long term aim of improving the level and efficiency of animal performance with respect to desired products and waste. The dynamic, mechanistic models developed in this study addressed specific aspects of rumen function which were not included in previous models, but have been recognized as being of major importance in the transformation of ingested to absorbed nutrients. In particular, the representation of microbial metabolism has been improved. Results of model evaluation showed that the outflow of nutrients from the rumen (NDF, starch, soluble sugars, nitrogen) was predicted well on a wide range of dietary inputs. However, the type of VFA was not predicted satisfactorily. Therefore, an experiment was conducted to study absorption rates of VFA from the rumen. It was found that VFA absorption is affected to a different extent by rumen volume, pH and VFA concentration. These factors should be taken into account if production of individual VFA in the rumen is to be predicted accurately. In another model developed in the present study, major aspects of rumen protozoal metabolism have been represented mathematically. The mathematical integration of protozoal, bacterial and dietary characteristics provided a quantitative understanding of mechanisms of protozoal responses and their effects on nutrients available for absorption to changes in dietary inputs. The prediction of responses of the amount of product (milk, meat, wool) and the product composition should recognize metabolism of individual substrates within the rumen or available after absorption. The models developed in this study provide a basis for the estimation of the profile of nutrients available for absorption, but further consideration of the prediction of type of VFA formed in the rumen should have a high priority.
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GENERAL INTRODUCTION

GENERAL INTRODUCTION

The practical importance of research into feed utilization by domestic animals is obvious. The amount of feed and its composition in general, significantly affect the performance of the animal with respect to milk, meat, wool, and egg production and their composition, as well as the output of wastes. Feed is also the major single cost of animal production systems (De Boer & Bickel, 1988), and largely determines financial return to the farmer. Thus, it is important to consider the links between feed intake and composition, the cost of feed input, and the output of animal products. Hence, it is essential to understand the main mechanisms within the animal which underlie the responses to feed inputs.

In ruminants, the amount and type of nutrients available for absorption, and ultimately for production, generally differ largely from the profile of nutrients taken up. These differences result from the metabolic activities of rumen microorganisms. Microbial material (particularly microbial protein) and the endproducts of rumen fermentation (particularly volatile fatty acids), represent a major part of the total available nutrient supply. For example, in a classic study Virtanen (1966) showed that cows, on diets lacking true protein, maintained milk production. In dairy cattle, volatile fatty acids (VFA) constitute the major source of energy, providing at least 50% of the total digested energy (Sutton, 1985). Considerable research on various aspects of rumen fermentation processes, has accumulated over time and relevant reviews include the proceedings of the most recent symposia on ruminant metabolism and physiology (McDonald & Warner, 1975; Ruckebusch & Thivend, 1980; Milligan *et al.* 1986; Tsuda *et al.* 1991). The response of the profile of nutrients available for absorption to variations in nutrients entering the rumen, is the result of complex interactions which occur during the fermentation process. However, by concentrating research on the individual components of the rumen fermentation, rather than on its integration, has resulted in insufficient information on many of the important mechanisms linking the individual components (Gill *et al.* 1989), and has hampered adequate predictions concerning the supply of absorbed nutrients (Beever, 1984). Examples of such interactions in the rumen, are the responses of organic matter digestibility to an increase in the protein content of low protein diets at low or high intake levels (Oldham, 1984); the fermentation or incorporation of amino acids related to the availability of energy (Russell *et al.* 1983); and the response in the glucogenic to non-glucogenic VFA ratio in rumen fluid, to the source of energy and protein (McCarthy *et al.* 1989).

In parallel with the increase in our understanding of individual components of rumen fermentation processes, there has been an increasing interest and awareness in the construction and value of mathematical models, aimed primarily at providing an integration of existing knowledge (Baldwin *et al.* 1970). This has led to subsequent refinements and updates as more information became available (Baldwin

et al. 1977; Beever *et al.* 1981; Murphy *et al.* 1986). At the same time, there have been efforts to improve the mathematical representation of some of the processes (France *et al.* 1982). On this basis, Baldwin *et al.* (1987) reported a model of digestion in the rumen of the lactating dairy cow, as an essential prerequisite of a model of whole body metabolism.

The main aim of the present study is to model the rumen fermentation processes, to achieve the integration and quantification of these processes, with the long term aim of improving significantly the level and efficiency of animal performance with respect to desired products and waste. The application of mathematical modelling to solving biological problems has been described in a number of authorative texts (for modelling in agriculture see France & Thornley, 1984; Thornley & Johnson, 1990), while the application of models to ruminant digestion and metabolism can be found in the proceedings of relevant workshops (Baldwin & Bywater, 1984; Robson & Poppi, 1990). Briefly, mathematical modelling can be applied (i) to identify gaps in current knowledge, thus stimulating new ideas and experimental approaches; (ii) to aid in the evaluation of hypotheses, expressed in mathematics, providing a quantitative description and understanding of mechanisms within a system; (iii) to predict the response of a system (tissue, organs, organisms, etc.) to changes in input to the system. In the present study, whole rumen function has been mathematically described using dynamic, deterministic, mechanistic models. The basic terminology, hierarchy, and principles in this study follow the lines described by France & Thornley (1984).

Over past decades, considerable progress in the modelling of rumen function has been made, due to the increased availability of data and power of computers, but above all due to the improved concepts that have been formulated as knowledge of the subject increased. Thus, for a number of justifiable reasons, the earlier models could not include certain aspects of rumen function which are presently recognized as being of major importance in the overall transformation of ingested nutrients to absorbed nutrients. These aspects have been highlighted by Beever (1984) and Sauvant (1988), and the mathematical representation of these aspects has been reviewed briefly by Dijkstra *et al.* (1990). In Chapter 1, a mathematical model is described, that simulates the digestion, absorption and outflow of nutrients in the rumen of cattle. This model addressed those specific problems, which had not been satisfactorily resolved to date. The mathematical procedures used in the model and the level of aggregation within the model were similar to those advanced previously (France *et al.* 1982; Baldwin *et al.* 1987). Key elements in the model described in Chapter 1, which previous models either did not or did not adequately represent, included microbial substrate preference, differential outflow of rumen microbes, variable microbial composition, recycling of microbial biomass within the rumen, significance of microbial distribution within the rumen, uncoupling of fermentation with respect to nitrogen availability, and pH control of microbial cellulolytic activity and of VFA and ammonia absorption. Given the specific issues being identified and

subsequently addressed, this model represented a substantial change from those which have been published earlier. The validity of these improvements and the prediction of nutrient supply on a variety of dietary inputs, are described in a companion paper (Chapter 2).

The results of model evaluation (Chapter 2) stress the need for quantitative information on the rate of absorption of individual VFA from the rumen. Several experiments have been performed to study this absorption rate (reviews Bugaut, 1987; Bergman, 1990). However, the utilization of the results for VFA absorption rates, in order to estimate the amounts of individual VFA available from fermentation in cattle, has been severely hampered by the confounding between pH and VFA concentration effects, the general inapplicability of *in vitro* results on VFA absorption to the normal functioning ruminant, and the fact that almost all experiments have been performed with sheep or using rumen tissue of sheep. In Chapter 3, an experiment is described, which was performed to quantify the effects of rumen liquid volume, pH and concentration of VFA on the fractional absorption rates of acetic, propionic and butyric acid from the rumen of lactating dairy cows. The results of this study can be used to predict accurately the production of individual VFA from substrate fermentation in the rumen.

The impact of protozoa on the fermentation processes in the rumen, and on the supply of nutrients to the ruminant has been investigated by many research workers. There is considerable debate on the role of the rumen protozoa in these processes (see reviews Jouany *et al.* 1988; Williams & Coleman, 1988). Although the importance of rumen protozoa in the transformation of ingested to absorbed nutrients is recognized in models of rumen fermentation (Reichl & Baldwin, 1976; France *et al.* 1982; Murphy *et al.* 1986; Russell *et al.* 1992), the explicit representation of protozoal metabolism has received only limited attention (Dijkstra *et al.* 1990). The model described in Chapter 1 represented protozoal predation on bacteria and selective retention of protozoa within the rumen. However, considerable simplifications had to be made, and evaluation of the model (Chapter 2) indicated the need to represent protozoal metabolism and interactions (commensalism, predation, competition) with bacteria in more detail. Mathematical procedures to describe these interactions have not been developed satisfactorily, however (Bazin, 1981), and particularly not for the rumen where quantitative data on the mechanisms associated with these interactions are scarce (Beever *et al.* 1986). Thus, specific emphasis was placed on the mathematical representation of the metabolic activities of rumen protozoa and their interactions with bacteria, based on data and hypotheses from the literature. This resulted in the development of a model modified from that described in Chapter 1, which allowed the evaluation of concepts and data in order to provide a quantitative understanding of the protozoal dynamics of the rumen, and of the integration of protozoal functioning with other microorganisms and with composition of the diet (Chapter 4). A specific application of this model, is the establishment of the role of rumen protozoa in fibre degradation for a wide range of dietary inputs

(Chapter 5). This is of particular interest in view of the increased interest and utilization of roughages for dairy and beef cattle herds in the Netherlands, and considering the widespread use of high fibrous diets in many developing countries.

In the General Discussion, the major findings of Chapters 1 - 5 are discussed. An important consideration in the Discussion, is the integrative character of the processes in the rumen. The future application of the results for the prediction of nutrient supply is discussed also. The contrast between current feed evaluation systems and the mechanistic models of nutrient supply as affected by the complex processes within the rumen are particularly emphasised, and there is an assessment of the implications for research efforts and prospects for the prediction of the responses of cattle to changes in amount and composition of feed fed.

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Chapter 1

SIMULATION OF NUTRIENT DIGESTION, ABSORPTION AND OUTFLOW IN THE RUMEN: MODEL DESCRIPTION

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SIMULATION OF NUTRIENT DIGESTION, ABSORPTION AND OUTFLOW IN THE RUMEN: MODEL DESCRIPTION

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A mathematical model is described that simulates the digestion, absorption and outflow of nutrients in the rumen. The model consists of 17 state variables, representing nitrogen, carbohydrate, lipid, microbial and volatile fatty acid pools. The flux equations are described by Michaelis-Menten or mass action forms with parameters calculated from the literature. Several specific areas of improvement in representation of rumen processes were reconsidered during model development. These included microbial substrate preference, differential outflow and chemical composition of rumen microbes, recycling of microbial matter within the rumen, uncoupling of fermentation with respect to nitrogen availability, reduced microbial activity at reduced rumen pH and pH-dependent absorption of volatile fatty acids and ammonia. The model was used to examine the effects of the diet on the profile of nutrients available for absorption and was shown to respond appropriately to different intake and nitrogen levels. The validity of the improvements and the predictions of nutrient supply on a variety of dietary inputs are tested in a companion paper.

Rumen: Computer simulation: Mathematical model: Ruminants: Microbial metabolism

Both the importance and complexity of rumen fermentation and its impact on nutrient supply have been recognized, and considerable research evidence has accumulated on various aspects. Attempts to predict the supply of nutrients by statistical analysis of data sets have been of limited value (Beever, 1984). Thus, there has been an increasing interest and awareness in the construction and value of mathematical, mechanistic models of rumen fermentation, intended primarily to integrate existing knowledge (Baldwin *et al.* 1970). Models of rumen fermentation have been continuously refined biologically (Baldwin *et al.* 1977; Black *et al.* 1981) and mathematically (France *et al.* 1982), and recently a model of digestion in the rumen of lactating dairy cows was described (Baldwin *et al.* 1987). Whilst none of these models can be considered to adequately predict the outcome of rumen fermentation in all dietary situations, their general behaviour has been encouraging, and, through a critical re-evaluation of both concepts and data availability, have had an impact on subsequent research effort in this area. However, a number of specific weaknesses have been identified. Beever *et al.* (1981) were unable to adequately predict duodenal protein supply from low protein diets, whilst Baldwin *et al.* (1987) encountered problems in predicting the rumen digestibility of fibre from high grain containing diets.

All models had some difficulties in accurately predicting the yield of individual volatile fatty acids.

The present paper describes a mathematical model of nutrient digestion in the rumen and nutrient absorption and outflow from the rumen, based on concepts advanced by France *et al.* (1982) and Baldwin *et al.* (1987), but in which a number of the specific issues identified as being insufficiently represented in earlier models were addressed. In particular, aspects relating to microbial recycling, microbial substrate preference, uncoupled microbial fermentation, effect of pH on microbial activity and volatile fatty acid (VFA) and ammonia absorption were considered, and, taking account of both amylolytic and fibrolytic microbes, variation in microbial chemical composition, as influenced by microbial species and nutrient availability, was addressed. The primary objective of the model was to examine the effect of supplementation of forage diets on the profile of nutrients available for absorption in cattle. The mathematical description, assumptions made and model parameterization are described in this paper; a companion paper presents results of sensitivity analyses and comparisons of experimental data with simulated values for a range of dietary treatments (Neal *et al.* 1992).

THE MODEL

The complete model is summarized in Figure 1, and principal fluxes for grouped nutrients are shown in Figure 2. The equations that constitute the model, the abbreviations used to define the entities in the model and the variables and parameters describing the properties of the model are listed in the Appendix. All pools are expressed in moles except for the microbial pools, which are in grams. To describe the feed entities in molar terms, average molecular masses of monomers of protein, carbohydrates and lipid are assumed to be 110, 162 and 675 g, respectively. Volume is expressed in litres (l) and time in days (d). The flux equations are described by Michaelis-Menten or mass-action forms. Parameters of the Michaelis-Menten equations are given in Table 1; yield, requirement and fraction parameters in Table 2; stoichiometric yield parameters in Table 3 and microbial growth requirements in Table 4. The application of Michaelis-Menten equations in animal biology and biochemistry, including the application to studies of transport of molecules and ions across cell membranes and to studies of microbe and cell cultivation, is generally acknowledged in modelling (Gill *et al.* 1989).

The assumption made is that rumen metabolism depends only on the carbon-containing (hexoses) and nitrogen-containing (ammonia or protein) containing substrates, with other nutrients (such as minerals and vitamins) assumed to be present in nonlimiting amounts. Hexoses are the products from hydrolysis of soluble sugars, starch, pectin, cellulose or hemicellulose. Two microbial pools, representing three microbial groups, were distinguished according to microbial substrate

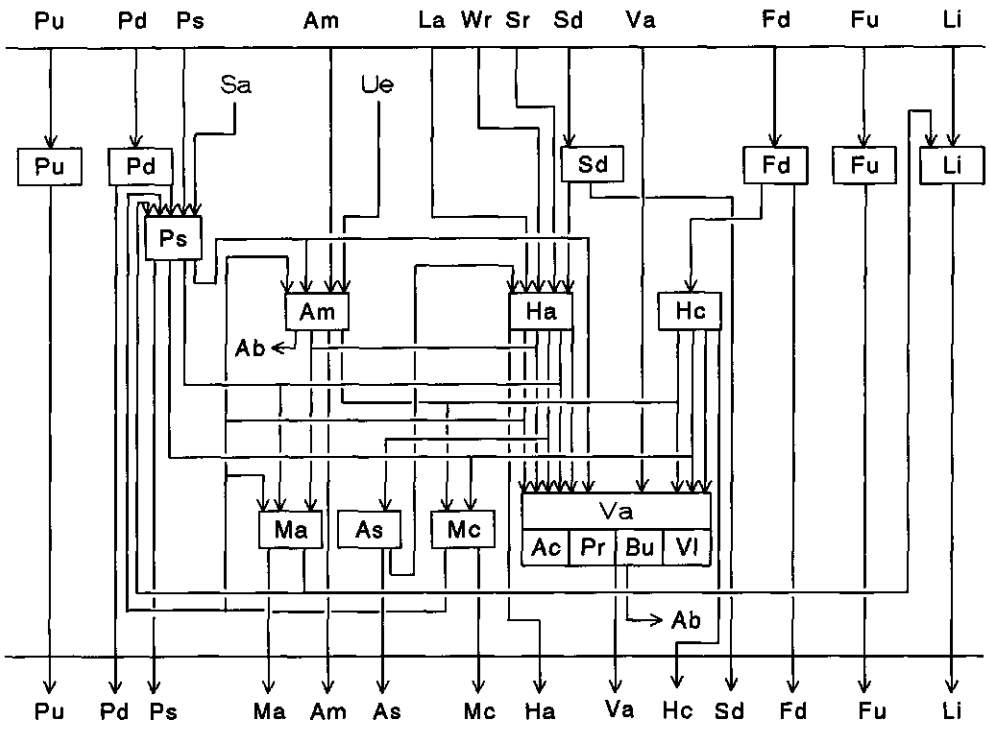

Figure 1. Diagrammatic representation of rumen model. Boxes enclosed by solid lines indicate state variables; arrows indicate fluxes. Abbreviations used: Ac, Acetic acid; Am, Ammonia; As, Amylolytic microbial storage polysaccharides; Bu, Butyric acid; Fd, Rumen-degradable neutral detergent fibre; Fu, Rumen-undegradable neutral detergent fibre; Ha, Hexose available to amylolytic microbes; Hc, Hexose available to fibrolytic microbes; La, Lactic acid; Li, Lipids; Ma, Amylolytic microbial mass; Mc, Fibrolytic microbial mass; Pd, Rumen-degradable protein; Pr, Propionic acid; Ps, Rumen fluid-soluble protein; Pu, Rumen-undegradable protein; Sa, Saliva; Sd, Rumen-degradable starch; Sr, Rumen fluid-soluble starch; Ue, Urea; Va, Volatile fatty acids; Vl, Valeric acid; Wr, Water-soluble carbohydrates.

preference: a pool of amylolytic microbes (bacteria and protozoa), which utilized hexose derived from nonstructural carbohydrates (soluble sugars, starch and pectin), and fibrolytic microbes, which utilized hexose derived from structural carbohydrates (cellulose and hemicellulose). In the absence of specific data to calculate rate constants for amylolytic and fibrolytic microbes, rate constants were assumed to be equal for both types of microbes unless otherwise stated.

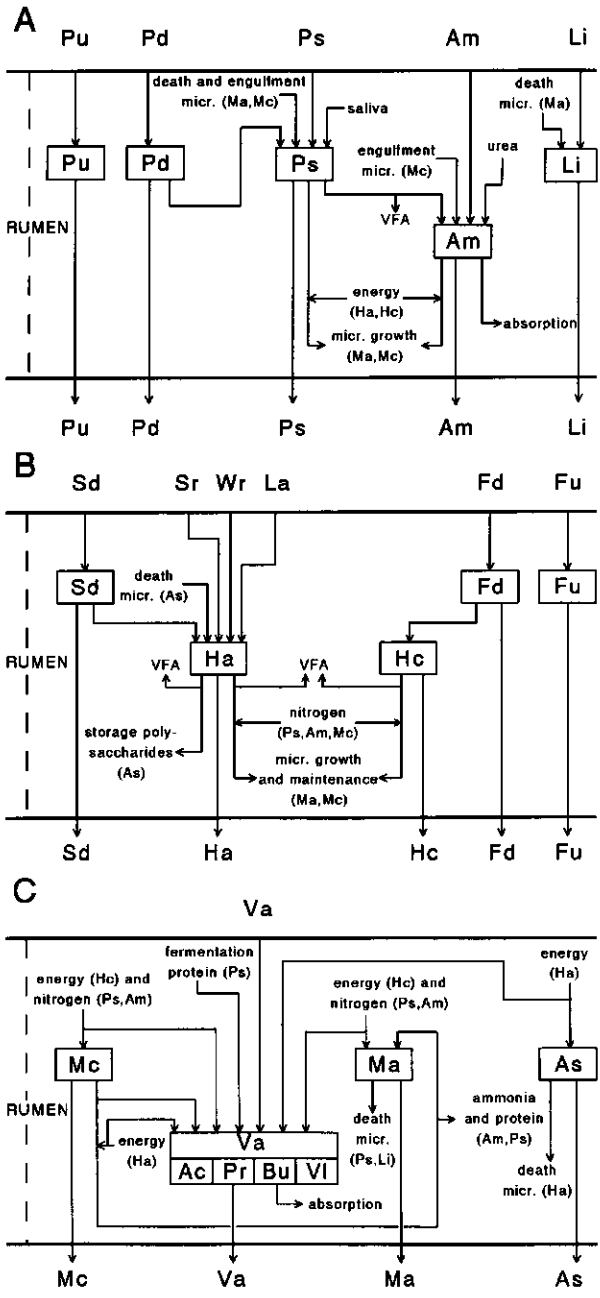

Figure 2. Diagrammatic representation of rumen model with respect to input and output of protein, ammonia and lipid (A), fibre, starch and hexose (B) and microbial matter and volatile fatty acids (C). Boxes enclosed by solid lines indicate state variables; arrows indicate fluxes. See Figure 1 for key to abbreviations.

PARAMETERIZATION

Input parameters

The amount of dietary nutrients entering the rumen pools is derived from the chemical description of the diet and the amount fed to the animal. Nitrogen-containing fractions of the diet include rumen-degradable, soluble (Ps) and insoluble (Pd) protein, undegradable protein (Pu) and ammonia (Am). The carbohydrate fractions of the diet are rumen-degradable (Fd) and undegradable (Fu) neutral detergent fibre, rumen-degradable, soluble (Sr) and insoluble (Sd) starch and water-soluble carbohydrates (Wr). All soluble entities are assumed to be completely degradable. Furthermore, lipid (Li) and organic acids: lactate (La), acetate (Ac), propionate (Pr), butyrate (Bu) and valerate (Vl) are considered, the latter being included to accommodate fermented feeds such as ensiled forages. These inputs did not change with the progress of time, thus simulating rumen fermentation processes during the day in a frequently fed animal. The soluble and insoluble, degradable and undegradable components of dietary protein or carbohydrates can be determined by the nylon-bag technique (Nocek & Grant, 1987; Tamminga *et al.* 1990) and will allow estimates of digestion turnover time (the reciprocal of the degradation rate constant) of NDF (T_{Fd}), starch (T_{Sd}) and protein (T_{Pd}) to be calculated. These are used to adjust for differences

Table 1. *Parameter values*

Transaction	$M_{i,jk}$ or $M^*_{i,jk}$	T^*_i	v^*_{jk}	$M_{Ha,jk}$ or $M_{Hc,jk}$	$M_{pH,jk}$	$J_{Am,jk}$	$J_{Ps,jk}$	$J_{Ha,jk}$ or $J_{Hc,jk}$	$J_{pH,jk}$	$\Phi_{pH,jk}$
Ac,AcAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48
Am,AmAb	0.0132	-	1.10	-	7.5	-	-	-	-	7.85
Am,AmMa	0.00135	-	0.0528	0.0159	-	-	-	-	-	-
Am,AmMc	0.00135	-	0.0528	0.0159	-	-	-	-	-	-
Am,UeAm	-	-	0.00165	-	-	0.00621	-	-	-	-
Bu,BuAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48
Fd,FdHc	0.332	0.83	0.1646	-	5.97	-	-	-	-	22.9
Ha,HaAs	0.0268	-	0.053	-	-	-	-	-	-	-
Ha,HaVa	0.055	-	0.1646	-	-	0.00861	0.01465	-	-	-
Hc,HcVa	0.055	-	0.1646	-	-	0.00861	0.01465	-	-	-
Mc,McEg	34.694	-	15.439	-	-	-	-	-	-	-
Mc,McAm	-	-	-	-	-	-	-	0.0165	-	-
Mc,McMa	-	-	-	0.0248	-	-	-	-	-	-
Pd,PdPs	0.264	0.66	0.0576	-	-	-	-	-	-	-
Pr,PrAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48
Ps,PsAm	0.0289	-	0.0144	-	-	-	-	0.0165	-	-
Ps,PsMa	0.0224	-	0.0576	0.0248	-	-	-	-	-	-
Ps,PsMc	0.0224	-	0.0576	0.0248	-	-	-	-	-	-
Sd,SdHa	0.416	1.04	0.2179	-	-	-	-	-	-	-
Vl,VlAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48

See Appendix for explanation of notation.

Table 2. *Yield, requirement and fraction parameters*

Transaction	$Y_{i,jk}$	$R_{i,jk}$	$f_{j,k}$ or $f_{i,jk}$	Transaction	$Y_{i,jk}$	$R_{i,jk}$	$f_{j,k}$ or $f_{i,jk}$
Ac,InAc	0.0167	-	-	Hf,HaAs	-	-	0.306
Am,InAm	0.0588	-	-	Hf,McMa	-	-	0.711
Am,McAm	0.0084	-	-	Hf,PsMa	-	-	0.711
Am,PsAm	1.257	-	-	Hf,PsMc	-	-	0.711
Am,UeAm	2.0	-	-	-,LcLe	-	-	2.250
As,HaAs	112.5	-	-	Li,InLi	0.0015	-	-
Bu,InBu	0.0114	-	-	Li,MaMd	0.00021	-	-
Fd,InFd	0.0062	-	-	Ma,AmMa	118.91	-	-
Fu,InFu	0.0062	-	-	Ma,McMa	149.48	-	-
Ha,AmMa	-	1.793	-	Ma,PsMa	149.48	-	-
Ha,LaHa	0.0025	-	-	Mc,AmMc	118.91	-	-
Ha,MaMd	0.0062	-	-	Mc,PsMc	149.48	-	-
Ha,McMa	-	0.0086	-	Pd,InPd	0.0091	-	-
Ha,PsMa	-	1.291	-	Pr,InPr	0.0135	-	-
Ha,SdHa	1.0	-	-	Ps,InPs	0.0091	-	-
Ha,SrHa	0.0062	-	-	Ps,MaMd	0.0067	-	-
Ha,WrHa	0.0062	-	-	Ps,McPs	0.0067	-	-
Hc,AmMc	-	1.793	-	Ps,PdPs	1.0	-	-
Hc,FdHc	1.0	-	-	Ps,SaPs	0.0010	-	-
Hc,PsMc	-	1.291	-	Pu,InPu	0.0091	-	-
Hf,AmMa	-	-	0.526	Sd,InSd	0.0062	-	-
Hf,AmMc	-	-	0.526	Vl,InVl	0.0098	-	-

See Appendix for explanation of notation.

between feed components in availability for hydrolysis. The fractional outflow rates of both the fluid (k_{FlEx}) and solid phase (k_{SoEx}) are specified in the model, and if no suitable rates are available from experiments, these passage rates as well as rumen volume can be estimated using the multiple regression equations given by Owens & Goetsch (1986).

The fraction of protozoa in the amylolytic microbial pool ($f_{Po,Ma}$), an input parameter to the model, is assumed to be 0.35, estimated from microbial outflow from the rumen reported by Firkins *et al.* (1987) and Siddons *et al.* (1984, 1985) and is held constant during each run, after correction according to the amount of lactate in the feed and the expected pH of rumen fluid. Considering the substantial contribution of protozoa to the ruminal catabolism of lactate (Chamberlain *et al.* 1983), $f_{Po,Ma}$ in presence of lactate in the feed is calculated as:

$$f_{Po,Ma} = [0.35(f_{St,In} + f_{Wr,In}) + 0.9x0.4f_{La,In}]/(f_{St,In} + f_{Wr,In} + 0.4f_{La,In})$$

where $f_{St,In}$, $f_{Wr,In}$ and $f_{La,In}$ are the fractions of starch, water-soluble sugars and lactate in the feed, respectively, the 0.9 term indicates the major contribution of

protozoa to lactate metabolism and 0.4 is the corrected energy equivalent of lactate compared with hexose. In view of the intolerance to low pH values (< 6.0) shown by many rumen protozoa (Eadie *et al.* 1970), $f_{Po,Ma}$ is decreased proportionally for pH values less than 6.0. The pH value of the rumen fluid and minimum pH reached during the day are also parameters in the model, affecting the degradation rate of fibre and absorption rates of ammonia and VFA.

Rate constants

Microbial pools, general

In view of specific substrate preference (Russell, 1984), the microbial population is represented by two pools, comprising amylolytic microbes (Ma), utilizing hexose derived from nonstructural carbohydrates (Ha), and fibrolytic microbes (Mc), utilizing hexose derived from structural carbohydrates (Hc). The necessity of such a representation has been shown previously by Baldwin *et al.* (1987). However, for the present modelling exercise, this substrate preference is combined with other characteristics such as recycling of microbial matter, differential outflow from the rumen and variation in microbial composition.

Jouany *et al.* (1988) and others suggested that microbial recycling can significantly affect both energy and protein utilization in the rumen and ultimately nutrient supply to the ruminant. Recycling of microbial matter is mainly associated with protozoal predation (Wallace & McPherson, 1987). The effect of engulfment of bacteria by protozoa has been evaluated in the linear programming model of Reichl & Baldwin (1976). On grounds of complexity, insufficient representation of hypotheses and inability to parameterize the model, these authors concluded that additional concepts regarding microbial interactions were required. Equally, Black *et al.* (1981) stated that their representation of microbial recycling was in need of major reappraisal. In the present model, protozoal characteristics such as selective retention and lysis of protozoa (Jouany *et al.* 1988) have been included. The major sources of carbon for energy and growth for protozoa are starch and soluble sugars; thus, the protozoa are included in the amylolytic microbial pool.

Several authors have reported markedly different microbial chemical compositions (review Storm & Ørskov, 1983), with storage polysaccharides being the most variable component. Such variation in microbial composition can significantly affect both the amount and type of nutrients passing out of the rumen and equally has important effects on the energy and nitrogen metabolism of the microbes (Hespell & Bryant, 1979). Thus, in contrast to the fixed chemical composition of microbial dry matter (DM) assumed in previous rumen models, the carbohydrate content of the microbes is allowed to vary with rumen availability of nitrogen and energy. The carbohydrate content of microbial dry matter is low in the rumen of animals fed low quality roughage diets, in which the majority of the microbes are expected to be of the fibrolytic type (McAllan & Smith, 1977). Hence, in the present model storage polysaccharides are linked to amylolytic microbes and are thus named amylolytic

storage polysaccharides (As). The polysaccharide-free microbial dry matter of both amylolytic and fibrolytic microbes is assumed to be constant and is given in Table 4 (Czerkawski, 1976; Hespell & Bryant, 1979; Merry & McAllan, 1983; Storm & Ørskov, 1983) along with the estimated amounts of hexose and either protein or ammonia required for biosynthesis of microbial cells (Stouthamer, 1973; Hespell & Bryant, 1979). It is assumed that hexose fermentation yields 4.5 mol ATP/mol hexose (Russell & Wallace, 1988), that production of reduced cofactors during hexose catabolism is sufficient to meet the demands of biosynthesis, that microbial lipid is derived only from hexose and finally that all nucleic bases are synthesized in equal amounts. As discussed by Hespell & Bryant (1979), no correction for the amount of energy required for transport processes has been made.

Amylolytic microbial pool, Ma

There are three inputs to the amylolytic microbial pool, related to the nitrogen source used for growth: ammonia, soluble protein and protein from engulfed microbial matter (Eq. 11.2 to 11.4). Microbial yield per unit nitrogen source expended can be calculated from the chemical composition shown in Table 4. Only fibrolytic microbes are assumed to be engulfed by the protozoa (Eq. 11.4). This is a simplification related to the level of aggregation adopted in the model and in light of the problems encountered previously in developing models designed to examine interrelationships among species (Dijkstra *et al.* 1990). Engulfed microbial protein can be incorporated into protozoal cells, fermented to VFA and ammonia or released into the rumen fluid (Coleman & Sandford, 1979), and is represented in a balance equation (Eq. 2.13). This is achieved by calculating the amount of engulfed microbial protein released into the rumen fluid as the difference between microbial protein engulfed and the amount of engulfed protein incorporated in protozoal cells or fermented. It is assumed that the amount of engulfed bacterial protein incorporated into protozoal cells depends on the availability of energy (Ha), equivalent to the utilization of protein for microbial growth (Eq. 2.7) explained in the soluble protein pool section.

The three outputs from the amylolytic microbial pool are related to their spatial distribution. Amylolytic bacteria are assumed to live free in rumen fluid and hence outflow is with the fluid (Eq. 11.6). The selective retention of protozoa reported by several authors (review Jouany *et al.* 1988) suggests that, in steady state, removal of protozoa to balance growth is mainly due to death and lysis of protozoa and only a relative small proportion of the protozoa is washed out to the omasum. Based on the data summarized by Faichney (1989), fractional outflow of protozoa from the rumen is assumed to be one half of the solid phase passage rate (Eq. 11.12), with the fractional death rate of protozoa assumed to be the difference between the fractional outflow rate of amylolytic bacteria and that of protozoa (Eq. 11.10).

Table 3. *Stoichiometric yield parameters for fermentation of carbohydrates and protein for roughage diets (> 50% roughage) and concentrate diets (< 50% roughage)*

	Roughage				Concentrate			
	Ac	Pr	Bu	Vl	Ac	Pr	Bu	Vl
Ce	1.32	0.17	0.23	0.03	1.58	0.12	0.06	0.09
He	1.13	0.36	0.21	0.05	1.12	0.51	0.11	0.07
Ps	0.40	0.13	0.08	0.33	0.36	0.16	0.08	0.33
St	1.19	0.28	0.20	0.06	0.80	0.60	0.20	0.10
Wr	1.38	0.41	0.10	0.00	0.90	0.42	0.30	0.04

See Appendix for explanation of notation; parameters adopted from Murphy (1984).

Amylolytic storage polysaccharides pool, As

The only input to this pool is from the utilization of amylolytic hexose (Eq. 12.2). The calculation of the rate constants involved will be described in the hexose section. Both amylolytic bacteria and protozoa are assumed to contain storage polysaccharides, the content varying with the amount of nonstructural carbohydrates available in relation to the nitrogen availability.

The three outputs from the amylolytic storage polysaccharide pool (Eq. 12.3 to 12.5) are equivalent to the outputs from the amylolytic microbial pool (Eq. 11.5 to 11.7).

Fibrolytic microbial pool, Mc

There are two inputs to the fibrolytic microbial pool, representing growth with hexose and either ammonia (Eq. 13.2) or soluble protein (Eq. 13.3) as nitrogen source. Two outputs from the fibrolytic microbial pool are represented; viz., engulfment by protozoa (Eq. 13.4) and washout from the rumen (Eq. 13.5). Fibrolytic microbes are known to adhere closely to particles (Cheng & Costerton, 1980) and hence are assumed to pass out of the rumen with the solid phase material. The equations for engulfment rates were obtained by transformation to Michaelis-Menten form of data presented in reciprocal plots of engulfment of mixed bacteria in vitro by *Epidinium ecaudatum* and *Entodinium spp.* (Coleman & Sandford, 1979). An equation for total engulfment rate (Eq. 13.4 and 13.7), corrected for the proportion of fibrolytic bacteria in the total bacterial biomass, was formed from the individual ones using the approach of Thornley (1976), taking the ratio of *Entodinia* to *Epidinia* and bacterial or protozoal numbers per gram of biomass reported by Warner (1962) and Eadie *et al.* (1970).

Degradable protein pool, Pd

The degradable protein pool receives input from the feed only (Eq. 1.2). Hydrolysis of insoluble protein by the proteolytic enzymes produced by the rumen

microorganisms is represented in Eq. 1.3. The maximum velocity of protein hydrolysis (v^*_{PdPs}) is assumed to be equal to the maximum rate of incorporation of hydrolysed protein into microbial cells (v^*_{PsMa} or v^*_{PsMc}), because protein fermentation rate is inversely related to energy availability. Calculation of the parameters associated with protein hydrolysis is considered in the next section. Degradable protein outflow from the rumen is calculated using the fractional outflow rate of solid phase material (Eq. 1.4).

Soluble protein pool, Ps

There are five inputs to the soluble protein pool: from the feed (Eq. 2.2), from saliva (Eq. 2.3), from hydrolysis of degradable protein (Eq. 2.4), from death and lysis of protozoa (Eq. 2.5) and from engulfed bacterial protein released into the rumen fluid (Eq. 2.6). The salivation rate (D_{Sa}, l/d) is calculated from data for cattle fed a range of diets (Bailey 1961; Cassida & Stokes, 1986). Ignoring the effects of feed dry matter concentration and particle size, a linear regression of dry matter intake and NDF content of the diet vs. salivation yields the equation:

$$D_{Sa} = 4.6 + 9.54\ DMI + 0.1357\ NDF \qquad (r^2 = 0.79)$$

where DMI is the dry matter intake (kg/d) and NDF the neutral detergent fraction in the feed (g/kg dry matter). The salivary protein content is assumed to be completely soluble and is calculated from the analysis of resting mixed saliva in non-lactating

Table 4. *Composition of polysaccharide-free microbial matter and amount of nutrients required for biosynthesis of microbial cell components: hexose for energy (Hx_{ATP}), hexose incorporated (Hx_{inc}), amino acids incorporated (Aa_{inc}) and ammonia incorporated (Am_{inc})*

Microbial cell component	Polysaccharide free dry weight	Biosynthesis from hexose and amino acids			Biosynthesis from hexose and ammonia		
		Hx_{ATP}	Hx_{inc}	Aa_{inc}	Hx_{ATP}	Hx_{inc}	Am_{inc}
	g/100 g			*mmol/g*			
Protein	53.0	5.35	-	4.81	5.72	4.08	6.07
DNA	3.4	0.16	0.15	0.37	0.57	0.23	0.46
RNA	12.3	0.45	0.51	1.22	1.48	0.77	1.54
Lipid	14.3	0.05	1.80	-	0.05	1.80	-
Cell wall	4.2	0.13	0.04	0.29	0.11	0.27	0.34
Ash	12.9	-	-	-	-	-	-
Total	100	6.14	2.50	6.69	7.93	7.15	8.41
Polysaccharide	-	2.72	6.17	-	2.72	6.17	-

See Appendix for explanation of notation.

cows as the difference between observed total nitrogen-content and urea nitrogen-content (Altman & Dittmer, 1968). The amount of protein from engulfed microbial matter released into the rumen fluid (Eq. 2.6) is assumed to be the difference between total bacterial protein engulfed and the amount incorporated in protozoal cells and fermented to ammonia (Eq. 2.13), as explained earlier.

There are five outputs from the pool: soluble protein incorporated in and fermented by amylolytic and fibrolytic microbes (Eq. 2.7 to 2.10) and outflow of soluble protein (Eq. 2.11). Most bacterial species are able to grow with ammonia as the sole source of nitrogen (Russell, 1984). Thus, ammonia uptake parameters were calculated from microbial growth parameters in ruminants fed protein-free diets at 2-h intervals and differing in urea content (Hume *et al.* 1970). At the highest urea content and hence highest rumen ammonia levels, and on the assumption that the maintenance requirement of microbes, containing 11.8% nitrogen in microbial dry matter, is 47 mg hexose/(g microbial dry matter·h) (Isaacson *et al.* 1975), application of the Pirt (1965) double reciprocal equation of microbial growth vs. dilution (i.e., growth) rate yields an estimate of maximum growth yield of 0.239 g microbial dry matter/g truly digested organic matter (OM). It is assumed that carbohydrates are by far the most important energy source for rumen microbes (Russell, 1984), and hence maximum uptake rate of energy equals maximum uptake rate of hexose. This in turn is calculated from data on soluble carbohydrate concentration in rumen fluid reported by Clapperton & Czerkawski (1969) in sheep fed a diet of chopped hay once daily. The peak value of soluble carbohydrate concentration in the rumen fluid at the end of the feeding time minus the pre-feeding value reported indicates the inability of microbes to utilize the offered amount of fermentable carbohydrates, i.e. maximum growth rate has been achieved. Assuming that the amounts of Fd, Wr and St in the diet are 49, 12.5 and 0%, respectively, of feed dry matter with rumen volume and concentration of microbial dry matter in the rumen of 5 L and 12.25 g/l, respectively (Siddons *et al.* 1985), and T_{Fd} equal to 0.69 d (Nocek & Grant, 1987), then maximum uptake rate of hexose is 1.1 g hexose/(g microbial dry matter·h). Hence, maximum uptake rate of ammonia (v^*_{AmMa} and v^*_{AmMc}) is 53 mmol ammonia/(g microbial dry matter·d).

Similarly, the uptake rate of ammonia by rumen microbes as influenced by ammonia concentration has been calculated, based on the other three diets differing in urea content reported by Hume *et al.* (1970), using nonlinear regression (SAS, 1985). Actual microbial growth with ammonia as nitrogen source is determined by the availability of hexose. In the absence of data on hexose concentration in rumen fluid in the experiment of Hume *et al.* (1970), an initial value of 2 mM is assumed for both types of hexose, based on pre-feeding values of contents of soluble sugars in rumen fluid reported by Clapperton & Czerkawski, (1969). Thus, the affinity constants $M_{Ha,AmMa}$ and $M_{Hc,AmMc}$ were estimated based on ammonia uptake rates of and parameters and initial values described above using nonlinear regression.

Once ammonia rate constants were established, soluble protein rate constants were calculated by applying a similar approach to data for animals consuming diets

containing contrasting levels and forms of nitrogen (Siddons *et al.* 1984 and 1985, Firkins *et al.* 1987). For each diet, the maximum growth yield was determined, ignoring the energy yielded by fermentation of protein and correcting for the energy yield of organic acids (Russell & Wallace, 1988). Next, the maximum growth with amino acids or peptides was calculated as the total maximum growth minus growth due to incorporation of ammonia. Maximum velocities of soluble protein incorporation (v^*_{PsMa} or v^*_{PsMc}) are assigned the highest values in the range found. In calculating the affinity constants $M_{Ps,PsMa}$ and $M_{Ps,PsMc}$, the amount of soluble protein in the rumen was estimated from the feed non-ammonia nitrogen (NAN) outflow, the fractional liquid outflow rate, the soluble fraction and the degradability of the insoluble fraction of the feed protein (Nocek & Grant, 1987; Erdman *et al.* 1987). Hexose affinity constants related to microbial growth on soluble protein were calculated as indicated for microbial growth with ammonia. The reference digestion turnover times of degradable fibre (T^*_{Fd}), starch (T^*_{Sd}) and protein (T^*_{Pd}) were assumed to be the average digestion turnover times applicable to diets used to calculate parameters associated with protein utilization; the affinity constants related to degradation of Fd, Sd and Pd were set arbitrarily at values given in Table 2.

Rate constants for protein fermentation were estimated from in vitro data with mixed rumen bacteria incubated with varying amounts of casein and carbohydrates (Russell *et al.* 1983). These authors found that in the presence of an adequate energy source, amino acids and peptides were incorporated into microbial cells, rather than fermented to ammonia and VFA. Thus, the value obtained without addition of carbohydrates, multiplied by 1.5 to correct for the difference in metabolism rate of amino acids observed in vivo and in vitro (Tamminga, 1979), yields the maximum velocity constant of protein fermentation (v^*_{PsAm}). The inhibition constants ($J_{Ha,PsAm}$ and $J_{Hc,PsAm}$) were then calculated on the basis of actual microbial growth and average ammonia concentration at different rates of carbohydrate addition reported by Russell *et al.* (1983), using nonlinear regression. Inhibition of protein fermentation by high ammonia levels was ignored because this occurs only at very high ammonia levels (Hespell, 1987). The affinity constant of soluble protein ($M_{Ps,PsAm}$) is calculated from the experiments described before.

Undegradable protein pool, Pu

There is only one input to the undegradable protein pool, viz., from the feed (Eq. 3.2) and the undegradable protein is assumed to pass out of the rumen at the same relative rate as the solid matter (Eq. 3.3).

Ammonia pool, Am

There are five inputs to, and four outputs from, the ammonia pool. The inputs are from feed (Eq. 4.2), urea transfer to the rumen (Eq. 4.3), fermentation of soluble protein in rumen fluid (Eq. 4.4 and 4.5) and protein from engulfed bacteria (Eq. 4.6). Calculations of the rate constants associated with protein fermentation and ammonia

incorporation have been explained earlier. The amount of ammonia produced by fermentation of engulfed bacterial protein was assumed to be related to hexose availability, i.e., a low level of amylolytic hexose will promote fermentation of engulfed microbial protein at the expense of incorporation of engulfed bacterial protein into protozoal cells. Owing to a lack of quantitative information, the value of the inhibition constant ($J_{Ha,McAm}$) is assumed to be the same as that for amylolytic hexose inhibition of soluble protein fermentation, calculated earlier. Transfer of urea to the rumen fluid both by saliva and via the rumen epithelium was considered, but in the absence of quantitative data on both routes, these were represented in one equation (Eq. 4.3). Urea transfer has shown to be affected by ammonia concentration in rumen fluid, plasma urea concentration and amount of organic matter digested in the rumen, the latter giving rise to microbial growth (Kennedy, 1980). Plasma urea concentration was assumed to be related to the nitrogen fraction of the feed. The rate constants in Eq. 4.3 and 4.12 were calculated from data on urea transfer reported by Kennedy (1980) for several diets fed to steers. Urea transferred to the rumen is assumed to be immediately hydrolysed to ammonia (Houpt, 1970).

Outputs from the ammonia pool are to amylolytic (Eq. 4.7) and fibrolytic bacteria (Eq. 4.8), absorption through the rumen wall (Eq. 4.9) and outflow from the rumen with the fluid (Eq. 4.10). Protozoa are not considered to incorporate ammonia for growth (Jouany *et al.* 1988). Absorption of ammonia through the rumen wall to the blood is assumed to be by simple diffusion, related to absorptive surface and the relative concentration of un-ionized ammonia (Hogan, 1961). Thus, ammonia absorption was represented as a function of ammonia concentration modified sigmoidally by pH of the rumen fluid (Eq. 4.13). Rate constants have been estimated using nonlinear regression, based on the ammonia absorption values reported by Hogan (1961) and Siddons *et al.* (1985).

Lipid pool, Li

There are two inputs to the lipid pool: from the feed (Eq. 5.2) and from death and lysis of protozoa (Eq. 5.3). The amount of lipid released by protozoal lysis ($Y_{Li,MaMd}$) can be calculated from the average chemical composition of microbial biomass (Table 4). The triglycerides in lipid are rapidly hydrolysed and outflow from the rumen is with the fluid phase (Eq. 5.4).

Degradable starch pool, Sd

Feed input to the degradable starch pool has been represented (Eq. 6.2). The degradable starch is either hydrolysed to amylolytic hexose (Eq. 6.3) or washed out from the rumen with the solid material (Eq. 6.4). The rate of degradable starch hydrolysis depends on the digestion turnover time of the starch in the diet (T_{Sd}) and the quantity of amylolytic microbes present. Because within-day patterns are not considered in the model, engulfment of starch particles by protozoa followed by digestion of these particles several hours later (Jouany *et al.* 1988) has been ignored.

Maximum velocity of starch hydrolysis (v^*_{SdHa}) is calculated from the uptake rate of hexose to achieve maximum microbial growth rates and formation of storage polysaccharides, assuming that microbes will not attack more starch than they can possibly utilize at maximum growth rate. The growth rate of polysaccharide-free microbial matter has been explained in the soluble protein pool section. Based on polysaccharide contents of microbial matter measured within 1 - 2 hours after feeding a concentrate diet to ruminants (McAllan & Smith, 1977) and the amount of hexose required per gram of microbial matter formed, maximum velocity of starch hydrolysis was calculated as 0.218 mol degradable starch/(g amylolytic biomass·d).

Degradable fibre pool, Fd

There is one input to the degradable fibre pool; from the feed (Eq. 7.2), and two outputs; to fibrolytic hexose (Eq. 7.3) and outflow from the rumen with the solid material (Eq. 7.4). Calculation of maximum rate of fibre hydrolysis (v^*_{FdHc}) was as explained earlier; however, in the model it is assumed that fibrolytic microbes do not contain storage polysaccharides. Hence, the value of v^*_{FdHc} is lower than v^*_{SdHa}. The rate of fibre hydrolysis, which is dependent on digestion turnover time of fibre (T_{Fd}) and amount of fibrolytic microbes, was modified sigmoidally by pH of the rumen fluid (Eq. 7.6). Except for the updated rumen model of Baldwin *et al.* (1987), described by Argyle & Baldwin (1988), none of the previous rumen models included such a modification. The rumen fluid pH value below which fibre digestion will be reduced was set at 6.3 (Erdman, 1988). Both the time below this critical pH value during the day (tf) and the minimum pH reached (pm) are input variables to the model. Based on the actual depression of cellulose or NDF digestion at low pH values reported by Terry *et al.* (1969), Stewart (1977), Hoover *et al.* (1984) and Shriver *et al.* (1986), $M_{pH,FdHc}$ and $\Phi_{pH,FdHc}$ were estimated using a nonlinear method. This representation is akin to that used in Argyle & Baldwin (1988). However, these authors could not challenge their representation. The validity of the present representation is tested in the companion paper (Neal *et al.* 1992).

Undegradable fibre pool, Fu

Like undegradable protein, this pool receives input from the feed (Eq. 8.2), and fractional outflow is equal to that of the solid matter (Eq. 8.3).

Amylolytic hexose pool, Ha

There are five inputs to and six outputs from the amylolytic hexose pool. Three of the inputs are directly from the feed, viz. water-soluble carbohydrates (Eq. 9.2), soluble starch (Eq. 9.3) and lactate (Eq. 9.4). The energy yield factor of hexose derived from lactate ($Y_{Ha,LaHa}$) is calculated from the molecular weight of lactate (90 g) and on the assumption that 1 mol of lactate fermented in the rumen yields 1 mol of ATP (Russell & Wallace, 1988). The other two inputs are from starch hydrolysis (Eq. 9.5) and from death and lysis of amylolytic microbes (Eq. 9.6), both explained earlier.

The six outputs from the amylolytic hexose pool are the requirements of hexose for microbial growth with ammonia (Eq. 9.7), with soluble protein (Eq. 9.8) and with engulfed bacterial protein (Eq. 9.9), hexose requirements for amylolytic storage polysaccharides formation (Eq. 9.10), hexose utilization for functions not related to growth (Eq. 9.11) and outflow with the fluid (Eq. 9.12). Requirement factors are given in Table 4. In contrast to other rumen models, no explicit maintenance energy requirement value has been used in the model. Rather, the total energy required for functions not directly related to growth, which are dependent on energy and nitrogen source availability, has been computed. This representation was chosen considering the highly variable maintenance energy requirement of individual bacterial species and the variable degrees of energetic uncoupling that occurs in bacteria (review Russell, 1984). The necessity for this representation becomes evident in the rate of degradation of energy-yielding substrates not having a relationship to the rate of microbial growth when growth is limited by the availability of some other substrate (Russell, 1984). In case of a lack of available nitrogen relative to the supply of available hexose, the potential of microbes to produce energy from catabolic processes is in excess of their potential to utilize that energy for biosynthetic purposes (Hespell & Bryant, 1979). Thus, the utilization rate ($U_{Ha,HaVa}$, eq. 9.11) of energy not related to growth is assumed to be the total energy substrate catabolism rate inhibited by the availability of ammonia and soluble protein. These inhibition constants were estimated by nonlinear regression, assuming that the difference between hexose uptake rate at theoretical maximum growth rate and hexose uptake rate at actual maximum growth rate, calculated from data as described in the soluble protein section, represents the hexose utilized for functions not related to growth. The protozoal component of Ma was assumed not to be limited by Ps concentration, because the concentration of protein within the protozoa is expected to be high due to engulfment of bacterial protein. The maximum rate of amylolytic hexose catabolism (v^*_{HaVa}) was assumed to be the hexose uptake rate required to achieve maximum microbial polysaccharide-free growth. The affinity constant ($M_{Ha,HaVa}$) is set at 55 mM, based on the microbial growth yields in the experiments mentioned previously. This representation will effectively increase the energy demand for non-growth related functions in situations where availability of nitrogen relative to hexose is low.

Fibrolytic hexose pool, Hc

There is one input to the fibrolytic hexose pool, which is from fibre hydrolysis (Eq. 10.2). There are four outputs: the requirement of fibrolytic hexose for microbial growth with ammonia (Eq. 10.3) and with soluble protein (Eq. 10.4), the utilization of fibrolytic hexose for microbial functions not related to growth (Eq. 10.5) and outflow of fibrolytic hexose with the solid material (Eq. 10.6), since fibrolytic microbes have been assumed to adhere closely to feed particles. Calculations of the rate parameters for fibrolytic hexose catabolism are similar to those for amylolytic hexose catabolism described in the previous section.

Volatile fatty acid pools, Ac, Pr, Bu and Vl

The inputs to and outputs from the acetic (Ac), propionic (Pr), butyric (Bu) and valeric (Vl) acid pools differ only in stoichiometric yield factors applied and will be considered together in this section. Twelve inputs to and two outputs from each of these pools have been represented. Inputs are from the feed, from fermentation of amylolytic or fibrolytic hexose related to growth with ammonia and soluble protein or to functions not related to growth, from fermentation of amylolytic hexose related to microbial growth with engulfed bacterial protein and related to biosynthesis of microbial storage polysaccharides, from fermentation of protein by amylolytic and fibrolytic microbes and from fermentation of engulfed bacterial protein. The hexose fermented as a fraction of total hexose utilized in a reaction can be calculated from Table 4. The amount produced from lactate fermentation was corrected for the carbon content equivalent ($f_{Lc,Le}$), because input of lactate to the amylolytic hexose pool was related to the energy content. Stoichiometric yield parameters (Table 3) for fermentation of individual substrates are given by Murphy (1984), adapted from Murphy *et al.* (1982) for roughage and concentrate diets. The yield factor of each VFA produced by amylolytic hexose fermentation is calculated from the ratio of amylolytic hexose production from lactate, starch and water-soluble carbohydrates, each multiplied by the corresponding stoichiometric yield parameter from Table 3, to the total amylolytic hexose production. It is assumed that stoichiometric yield parameters for lactate fermentation are equal to those for Wr fermentation. The yield factor of each VFA produced by fibrolytic hexose fermentation is calculated on the assumption that cellulose and hemicellulose behave as one nutritional entity.

The outputs from each VFA pool are outflow from the rumen with the fluids and absorption of VFA by simple diffusion (Hogan, 1961) through the rumen wall, represented by a function of individual VFA concentration and modified sigmoidally by rumen fluid pH. Rate constants are estimated using nonlinear regression, based on data on acetate absorption from the rumen reported by Hogan (1961) and Danielli *et al.* (1945). Although it is recognized that rate of absorption of VFA increases with chain length, this has been neglected because Murphy (1984) has taken the rate of absorption as proportional to concentration.

Model summary

The model is completely defined by equations in the Appendix. The differential equations of the seventeen state variables are integrated numerically for a given set of initial conditions and parameter values. The computer program was written in the simulation language ACSL (Advanced Continuous Simulation Language, Mitchell & Gauthier, 1981) and run on a VAX computer. Integration interval of $\Delta t = 0.001$ and 0.0025 d were used, with a fourth-order fixed-step-length Runge-Kutta method. The results presented were obtained by taking the predictions at 15 d. Solutions were not sensitive to integration method or interval in the stable region.

RESULTS AND DISCUSSION

This model has been used to study effects of dietary changes on nutrient supply to ruminants. The exogenous inputs to the model are feed intake and the components of these feeds. Hence a detailed description of the feed dry matter is required, but only a few reports fully characterize the feed in terms used in the model. Where information on some of the dietary constituents is lacking, the information can be obtained from tables of feed composition. Classification of the feed constituents into soluble and insoluble fractions, the latter separated into degradable and undegradable fractions, as well as digestion turnover times were based on nylon-bag incubations. Although qualitatively very similar, quantitative results reported in different papers on similar feedstuffs are highly variable (Van Straalen & Tamminga, 1989) and this will affect accuracy of model simulations, too. Clearly, future efforts to standardize these techniques is urgently required.

The model integrates current knowledge on factors affecting rumen function. From previous rumen modelling exercises, it became clear that representation of the metabolic activity of the rumen microbial population is of major importance in conversion of ingested nutrients to microbial biomass and VFA and hence in predicting the profile of available nutrients (Beever, 1984). In examining the profile of nutrients available for absorption, variation in efficiency of microbial growth as affected by dietary characteristics should be recognized. Thus, species differences in substrate utilization (Russell, 1984) and outflow rate from the rumen with the fluid or solid phase (Cheng & Costerton, 1980), recycling of microbial matter within the rumen (Jouany *et al.* 1988) and the chemical composition of microbial dry matter (Hespell & Bryant, 1979) will affect microbial growth efficiency, and these aspects have received considerable emphasis during development of the model. Equally, interactions between rumen function and host metabolism processes (e.g., urea transfer) will affect the profile of nutrients available for absorption, as will be discussed later. Although variations in microbial growth efficiency and hence nutrient supply to the animal have been demonstrated in vivo in numerous reports, understanding of the mechanisms involved has often been achieved by experiments performed in vitro. Complete sets of in vivo test data including detailed microbial metabolism and efficiency parameters are not available. Thus, evaluation of the model, of which representation of microbial metabolism is central, presents a problem. To a certain extent, model evaluation can be performed against data on nutrients available for absorption (including nutrients of microbial origin), as their prediction is an objective of the model, although this can give only indirect evidence on certain aspects of microbial metabolism represented in the model.

Several types of model evaluation are in order. Results of sensitivity analyses and comparisons of model estimates with experimental values on a wide range of diets, not used in model development, will be presented in the companion paper (Neal *et al.* 1992). In this paper, simulated values of nutrient supply at different intake and feed

nitrogen levels are compared with observed values obtained by Firkins *et al.* (1987), used to parameterize fermentation and growth rates of microbes and affinity, inhibition and velocity constants of protein, ammonia and hexose incorporation (Table 5). In the experiment steers were fed every 2 h diets containing 50% grass hay, 20% dry distillers grains and 30% concentrate at two levels of DMI (4.8 or 7.2 kg dry matter/d), and urea was infused continuously into the rumens of the steers at two levels (0.4 or 1.2% of diet dry matter). Although a number of parameters were derived from other sources, the model predictions closely match observations by Firkins *et al.* (1987). The reduced NDF digestion at the higher DMI level was simulated satisfactorily, as was the absolute amount of NDF escaping degradation in the rumen. Also, both the experimental and simulated values showed a lack of response in NDF digestion to urea level. No significant difference in microbial, non-microbial or total NAN outflow to the duodenum due to urea level was detected and simulations agree with these experimental data. Similarly, the increased total and microbial NAN outflow at the higher DMI level was simulated satisfactorily.

It is more difficult to evaluate the model predictions of VFA production in the rumen, because Firkins *et al.* (1987) neither determined VFA concentrations in rumen fluid nor estimated VFA production rates. It is striking that simulated molar proportions of individual VFA do not respond to DMI or urea levels. Likewise, in their companion study (Firkins *et al.* 1986), DMI level had no effect on molar proportions of individual VFA measured in vivo on hay-based diets, probably due to the frequent feeding system. Because the differences between treatments in digestion coefficients of fibre and starch were low in magnitude and the stoichiometric yield parameters associated with VFA production adopted from Murphy *et al.* (1982) do not account for a shift in type of VFA produced at decreased pH values other than providing separate stoichiometric parameter sets for roughage and concentrate diets, significant differences in simulated molar proportions of individual VFA were not expected.

The model overestimated the response in rumen ammonia concentration to the lower urea level, but showed no response to the increased feeding level. However, the response to DMI level was not significant in the experiment. Rumen ammonia concentration is the net result of ammonia production from feed, fermentation of protein or hydrolysis of urea, utilization by microbes, absorption through the rumen wall and passage out of the rumen. The processes of urea transfer across the rumen wall and in saliva and absorption of ammonia are influenced by the metabolism of nitrogen in the tissues of the animal. These metabolic processes are not represented within the model but stress the importance of recognizing interrelationships between ruminal processes and host tissue metabolism. Further, the parameters for the ruminal processes related to ammonia metabolism were derived from a number of in vivo and in vitro data sets. Thus, the apparently inaccurate quantitative simulation of rumen ammonia concentration may reflect our incomplete knowledge of all the factors affecting nitrogen transactions in the rumen.

Table 5. *Comparison of experimentally observed parameters (obs) and parameters predicted by the model (pred) for steers fed diets at low (4.8 kg dry matter/d) and high (7.2 kg dry matter/d) intake levels and at two levels of urea infused (0.4% or 1.2% of diet dry matter)*[1]

	Low		High		0.4%		1.2%	
	obs	pred	obs	pred	obs	pred	obs	pred
Duodenal flow of OM (g/d):								
Starch and sol. sugars	-	499	-	835	-	700	-	633
NDF	1203	1210	2009	2005	1591	1549	1588	1597
Total NAN	107	111	163	165	130	137	140	139
Microbial NAN	62	68	98	96	78	81	83	85
Non-microbial NAN	45	42	64	70	53	56	57	54
Ruminal digestion (%)								
Starch and sol. sugars	-	66.4	-	62.5	-	62.3	-	66.1
NDF	49.9	49.6	44.2	44.3	47.0	48.4	47.1	46.8
VFA production (mol/d)	-	18.0	-	24.7	-	21.8	-	21.6
VFA concentration (mM)	-	76.4	-	70.0	-	70.1	-	69.9
VFA molar proportions (mol/100 mol)								
Acetate	-	69.8	-	70.0	-	70.1	-	69.9
Propionate	-	15.3	-	15.4	-	15.3	-	15.3
Butyrate	-	10.3	-	10.2	-	10.3	-	10.2
Valerate	-	4.7	-	4.4	-	4.4	-	4.6
Ammonia concentration (mM)	4.8	4.0	5.3	3.8	3.6	2.2	6.6	6.2

NAN, non-ammonia nitrogen; NDF, neutral detergent fibre; VFA, volatile fatty acid.
[1] Observations from Firkins *et al.* (1987).

In conclusion, the model provides a mathematical representation of fermentation processes in the rumen to predict nutrient supply to ruminants and addresses limitations in representation of several processes occurring in previous models. The combination of features unique in the model described here are as follows: recognition of substrate preference of rumen microbes, linked to differential outflow from the rumen, the inclusion of protozoa to accommodate microbial recycling within the rumen and variation in microbial composition related to source and availability of hexose and nitrogen; the inclusion of functions representing energetic uncoupling related to nitrogen availability and functions representing the depressing effect of pH on fibre digestion; and the use of pH as a factor determining rate of absorption of ammonia and VFA. The model has been shown to simulate nutrient availability at different nitrogen and DMI levels. However, a full comparison between predicted and observed rumen fermentation parameters in data sets not used for model

development has been made to assess the validity of the improvements in representation of rumen processes made to predict the amount and type of nutrients available for absorption. This comparison is described in the companion paper (Neal *et al.* 1992).

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MATHEMATICAL APPENDIX

Table A1. *Symbols for entities and processes used in the model*

Symbol	Entity	Symbol	Entity
Ab	Absorption from rumen	Li	Lipids
Ac	Acetic acid	Ma	Amylolytic microbial mass
Am	Ammonia	Mc	Fibrolytic microbial mass
As	Amylolytic microbial storage polysaccharides	Md	Microbial death
Bu	Butyric acid	Ni	Nitrogen
Ce	Cellulose	Pd	Rumen-degradable protein
Eg	Engulfment of bacteria by protozoa	Po	Protozoa
Ex	Exit from rumen into lower tract	Pr	Propionic acid
Fd	Rumen-degradable neutral detergent fibre	Ps	Rumen fluid-soluble protein
Fe	Feed	Pu	Rumen-undegradableprotein
Fl	Rumen fluids	Ru	Rumen
Fu	Rumen-undegradable neutral detergent fibre	Sa	Saliva
Ha	Hexose available to amylolytic microbes	Sd	Rumen degradable starch
Hc	Hexose available to fibrolytic microbes	So	Rumen solids
He	Hemicellulose	Sr	Rumen fluid soluble starch
Hf	Proportion of hexose fermented for energy	St	Starch
In	Intake	Ue	Urea
La	Lactic acid	Va	Volatile fatty acids
Lc	Lactic acid carbohydrate equivalents	Vl	Valeric acid
Le	Lactic acid energy equivalents	Wr	Water-solublecarbohydrates

Table A2. *General notation used in model*

Notation	Translation	Units
C_i	Concentration of *i*	(mol or g *i*)/l
D_i	Driving variable with respect to *i*	(g or l *i*)/d
$f_{i,j}$	Fraction of *i* in *j*	g *i*/kg *j* or -
$f_{i,jk}$	Fraction of *i* in *j-k* transaction	-
$J_{i,jk}$	Inhibition constant for *j-k* transaction with respect to *i*	mol *i*/l or -
$k_{i,j}$	Mass action rate constant for *i-j* transaction	/d
$M_{i,jk}, M^*_{i,jk}$	Michaelis-Menten constants for *j-k* transaction with respect to *i*	(mol or g *i*)/l or -
$P_{i,jk}$	Rate of production of *i* by *j-k* transaction	(mol or g *i*)/d
$P_{mi,jk}$	Rate of production of *i* by *j-k* transaction due to *m* microbes	g *i*/d
Q_i	Quantity of *i*	mol or g *i*
$R_{i,jk}$	Requirement for *i* in *j-k* transaction	mol *i*/(mol or g *j*)
$\Phi_{i,jk}$	Steepness parameter associated with *i* for *j-k* transaction	-
t	Time	d
T_i	Digestion turnover time of feed component *i*	d
T^*_i	Reference digestion turnover time of feed component *i*	d
$U_{i,jk}$	Rate of utilization of *i* by *j-k* transaction	(mol or g *i*)/d
$U_{mi,jk}$	Rate of utilization of *i* by *j-k* transaction due to *m* microbes	mol *i*/d
V_i	Volume of *i*	l
v^*_{jk}	Maximum velocity for *j-k* transaction	(mol or g *j*)/(g or l)·d
v_{jk}	Velocity for *j-k* transaction	(mol or g *j*)/d
$v_{i,jk}$	Velocity for *j-k* transaction with respect to *i*	mol *j*/d
$Y_{i,jk}$	Yield of *i* for *j-k* transaction	(mol or g *i*)/(mol, g or l *j*)

i, *j*, *k* and *m* take values from Table A1.

Table A3. *Principal transactions occurring within the rumen*

Transaction	Substrate: principal; auxiliary	Products: principal; auxiliary
AmMa	Am; Ha	Ma; Ac, Pr, Bu, Vl
AmMc	Am; Hc	Mc; Ac, Pr, Bu, Vl
AsHa	As	Ha
FdHc	Fd	Hc
HaAs	Ha	As; Ac, Pr, Bu, Vl
HaVa	Ha	Ac, Pr, Bu, Vl
HcVa	Hc	Ac, Pr, Bu, Vl
MaPs	Ma	Ps
McPs	Mc	Ps
McAm	Mc; Ha	Am; Ac, Pr, Bu, Vl
McMa	Mc; Ha	Ma; Ac, Pr, Bu, Vl
PdPs	Pd	Ps
PsAm	Ps; Ha, Hc	Am; Ac, Pr, Bu, Vl
PsMa	Ps; Ha	Ma; Ac, Pr, Bu, Vl
PsMc	Ps; Hc	Mc; Ac, Pr, Bu, Vl
SaPs	Sa	Ps
SdHa	Sd	Ha
UeAm	Ue; Am	Am

See Tables A1 and A2 for explanation of notation.

Table A4. *Mathematical statement of model*

Protein and non-protein N digestion

Degradable protein pool, Q_{Pd} (mol)

Concentration:	$C_{Pd} = Q_{Pd}/V_{Ru}$	(1.1)
Input:	$P_{Pd,InPd} = Y_{Pd,InPd}D_{Pd}$	(1.2)
Outputs:	$U_{Pd,PdPs} = v_{PdPs}/[1 + M_{Pd,PdPs}/C_{Pd}]$	(1.3)
	$U_{Pd,PdEx} = k_{PdEx}Q_{Pd}$	(1.4)
Differential eq:	$dQ_{Pd}/dt = P_{Pd,InPd} - U_{Pd,PdPs} - U_{Pd,PdEx}$	(1.5)
Auxiliary eq:	$k_{PdEx} = k_{SoEx}$	(1.6)
	$M_{Pd,PdPs} = M^{*}_{Pd,PdPs}T_{Pd}/T^{*}_{Pd}$	(1.7)
	$v_{PdPs} = v^{*}_{PdPs}(Q_{Ma} + Q_{Mc})$	(1.8)

Soluble protein pool, Q_{Ps} (mol)

Concentration:	$C_{Ps} = Q_{Ps}/V_{Ru}$	(2.1)
Inputs:	$P_{Ps,InPs} = Y_{Ps,InPs}D_{Ps}$	(2.2)

(continued)

Table A4 *(continued). Mathematical statement of model*

	$P_{Ps,SaPs} = Y_{Ps,SaPs}D_{Sa}$	(2.3)
	$P_{Ps,PdPs} = Y_{Ps,PdPs}U_{Pd,PdPs}$	(2.4)
	$P_{Ps,MaMd} = Y_{Ps,MaMd}U_{Ma,MaMd}$	(2.5)
	$P_{Ps,McPs} = Y_{Ps,McPs}U_{Mc,McPs}$	(2.6)
Outputs:	$U_{MaPs,PsAm} = v_{Ma,PsAm}/[1 + M_{Ps,PsAm}/C_{Ps} + C_{Ha}/J_{Ha,PsAm}]$	(2.7)
	$U_{McPs,PsAm} = v_{Mc,PsAm}/[1 + M_{Ps,PsAm}/C_{Ps} + C_{Hc}/J_{Hc,PsAm}]$	(2.8)
	$U_{Ps,PsMa} = v_{PsMa}/[1 + M_{Ps,PsMa}/C_{Ps} + M_{Ha,PsMa}/C_{Ha}]$	(2.9)
	$U_{Ps,PsMc} = v_{PsMc}/[1 + M_{Ps,PsMc}/C_{Ps} + M_{Hc,PsMc}/C_{Hc}]$	(2.10)
	$U_{Ps,PsEx} = k_{PsEx}Q_{Ps}$	(2.11)
Differential eq:	$dQ_{Ps}/dt = P_{Ps,InPs} + P_{Ps,PdPs} + P_{Ps,MaMd} + P_{Ps,SaPs} + P_{Ps,McPs} - U_{MaPs,PsAm} - U_{McPs,PsAm} - U_{Ps,PsMa} - U_{Ps,PsMc} - U_{Ps,PsEx}$	(2.12)
Auxiliary eq:	$U_{Mc,McPs} = U_{Mc,McEg}[1 - 1/(1 + C_{Ha}/J_{Ha,McAm}) - 1/(1 + M_{Ha,McMa}/C_{Ha}]$	(2.13)
	$v_{Ma,PsAm} = v^{*}_{PsAm}Q_{Ma}$	(2.14)
	$v_{Mc,PsAm} = v^{*}_{PsAm}Q_{Mc}$	(2.15)
	$v_{PsMa} = v^{*}_{PsMa}Q_{Ma}$	(2.16)
	$v_{PsMc} = v^{*}_{PsMc}Q_{Mc}$	(2.17)
	$k_{PsEx} = k_{FlEx}$	(2.18)

Undegradable protein pool, Q_{Pu} (mol)

Concentration:	$C_{Pu} = Q_{Pu}/V_{Ru}$	(3.1)
Input:	$P_{Pu,InPu} = Y_{Pu,InPu}D_{Pu}$	(3.2)
Output:	$U_{Pu,PuEx} = k_{PuEx}Q_{Pu}$	(3.3)
Differential eq:	$dQ_{Pu}/dt = P_{Pu,InPu} - U_{Pu,PuEx}$	(3.4)
Auxiliary eq:	$k_{PuEx} = k_{SoEx}$	(3.5)

Ammonia pool, Q_{Am} (mol)

Concentration:	$C_{Am} = Q_{Am}/V_{Ru}$	(4.1)
Inputs:	$P_{Am,InAm} = Y_{Am,InAm}D_{Am}$	(4.2)
	$P_{Am,UeAm} = Y_{Am,UeAm}v_{Ue,Am}$	(4.3)
	$P_{MaAm,PsAm} = Y_{Am,PsAm}U_{MaPs,PsAm}$	(4.4)
	$P_{McAm,PsAm} = Y_{Am,PsAm}U_{McPs,PsAm}$	(4.5)
	$P_{Am,McAm} = Y_{Am,McAm}U_{Mc,McAm}$	(4.6)
Outputs:	$U_{Am,AmMa} = v_{AmMa}/[1 + M_{Am,AmMa}/C_{Am} + M_{Ha,AmMa}/C_{Ha}]$	(4.7)
	$U_{Am,AmMc} = v_{AmMc}/[1 + M_{Am,AmMc}/C_{Am} + M_{Hc,AmMc}/C_{Hc}]$	(4.8)
	$U_{Am,AmAb} = v_{AmAb}/[1 + M_{Am,AmAb}/C_{Am}]$	(4.9)
	$U_{Am,AmEx} = k_{AmEx}Q_{Am}$	(4.10)

(continued)

Table A4 *(continued). Mathematical statement of model*

Differential eq: $dQ_{Am}/dt = P_{Am,InAm} + P_{MaAm,PsAm} + P_{McAm,PsAm} + P_{Am,UeAm} + P_{Am,McAm} - U_{Am,AmAb} - U_{Am,AmMa} - U_{Am,AmMc} - U_{Am,AmEx}$ (4.11)

Auxiliary eq: $v_{UeAm} = v^{*}_{UeAm} V_{Ru} f_{Ni,Fe}/(1 + C_{Am}/J_{Am,UeAm})$ (4.12)

$v_{AmAb} = v^{*}_{AmAb} V_{Ru}^{0.75}/[1 + (M_{pH,AmAb}/pH)^{\Phi pH,AmAb}]$ (4.13)

$v_{AmMa} = v^{*}_{AmMa} Q_{Ma}(1 - f_{Po,Ma})$ (4.14)

$v_{AmMc} = v^{*}_{AmMc} Q_{Mc}$ (4.15)

$U_{Mc,McAm} = U_{Mc,McEg}/[1 + C_{Ha}/J_{Ha,McAm}]$ (4.16)

$k_{AmEx} = k_{FlEx}$ (4.17)

Lipid Digestion

Lipid pool, Q_{Li} (mol)

Concentration: $C_{Li} = Q_{Li}/V_{Ru}$ (5.1)

Inputs: $P_{Li,InLi} = Y_{Li,InLi} D_{Li}$ (5.2)

$P_{Li,MaMd} = Y_{Li,MaMd} U_{Ma,MaMd}$ (5.3)

Output: $U_{Li,LiEx} = k_{LiEx} Q_{Li}$ (5.4)

Differential eq: $dQ_{Li}/dt = P_{Li,InLi} + P_{Li,MaMd} - U_{Li,LiEx}$ (5.5)

Auxiliary eq: $k_{LiEx} = k_{FlEx}$ (5.6)

Carbohydrate digestion

Degradable starch pool, Q_{Sd} (mol)

Concentration: $C_{Sd} = Q_{Sd}/V_{Ru}$ (6.1)

Input: $P_{Sd,InSd} = Y_{Sd,InSd} D_{Sd}$ (6.2)

Outputs: $U_{Sd,SdHa} = v_{SdHa}/[1 + M_{Sd,SdHa}/C_{Sd}]$ (6.3)

$U_{Sd,SdEx} = k_{SdEx} Q_{Sd}$ (6.4)

Differential eq: $dQ_{Sd}/dt = P_{Sd,InSd} - U_{Sd,SdHa} - U_{Sd,SdEx}$ (6.5)

Auxiliary eq: $v_{SdHa} = v^{*}_{SdHa} Q_{Ma}$ (6.6)

$M_{Sd,SdHa} = M^{*}_{Sd,SdHa} T_{Sd}/T^{*}_{Sd}$ (6.7)

$k_{SdEx} = k_{SoEx}$ (6.8)

Degradable fibre pool, Q_{Fd} (mol)

Concentration: $C_{Fd} = Q_{Fd}/V_{Ru}$ (7.1)

Input: $P_{Fd,InFd} = Y_{Fd,InFd} D_{Fd}$ (7.2)

Outputs: $U_{Fd,FdHc} = v_{FdHc}/[1 + M_{Fd,FdHc}/C_{Fd}]$ (7.3)

$U_{Fd,FdEx} = k_{FdEx} Q_{Fd}$ (7.4)

Differential eq: $dQ_{Fd}/dt = P_{Fd,InFd} - U_{Fd,FdHc} - U_{Fd,FdEx}$ (7.5)

Auxiliary eq: $v_{FdHc} = v^{*}_{FdHc} Q_{Mc}[(1 - tf/24) + (tf/24)/[1 + (M_{pH,FdHc}/pm)^{\Phi pH,FdHc}]]$ (7.6)

(continued)

Table A4 *(continued). Mathematical statement of model*

$$M_{Fd,FdHc} = M^{*}_{Fd,FdHc} T_{Fd}/T^{*}_{Fd} \quad (7.7)$$

$$k_{FdEx} = k_{SoEx} \quad (7.8)$$

Undegradable fibre pool, Q_{Fu} (mol)

Concentration: $C_{Fu} = Q_{Fu}/V_{Ru}$ (8.1)

Input: $P_{Fu,InFu} = Y_{Fu,InFu} D_{Fu}$ (8.2)

Output: $U_{Fu,FuEx} = k_{FuEx} Q_{Fu}$ (8.3)

Differential eq: $dQ_{Fu}/dt = P_{Fu,InFu} - U_{Fu,FuEx}$ (8.4)

Auxiliary eq: $k_{FuEx} = k_{SoEx}$ (8.5)

Hexose available to amylolytic microbes, Q_{Ha} (mol)

Concentration: $C_{Ha} = Q_{Ha}/V_{Ru}$ (9.1)

Inputs: $P_{Ha,WrHa} = Y_{Ha,WrHa} D_{Wr}$ (9.2)

$P_{Ha,SrHa} = Y_{Ha,SrHa} D_{Sr}$ (9.3)

$P_{Ha,LaHa} = Y_{Ha,LaHa} D_{La}$ (9.4)

$P_{Ha,SdHa} = Y_{Ha,SdHa} U_{Sd,SdHa}$ (9.5)

$P_{Ha,MaMd} = Y_{Ha,MaMd} U_{As,MaMd}$ (9.6)

Outputs: $U_{Ha,AmMa} = R_{Ha,AmMa} U_{Am,AmMa}$ (9.7)

$U_{Ha,PsMa} = R_{Ha,PsMa} U_{Ps,PsMa}$ (9.8)

$U_{Ha,McMa} = R_{Ha,McMa} U_{Mc,McMa}$ (9.9)

$U_{Ha,HaAs} = v_{HaAs}/[1 + M_{Ha,HaAs}/C_{Ha}]$ (9.10)

$$U_{Ha,HaVa} = v_{HaVa}(1-f_{Po,Ma})/[\{1 + M_{Ha,HaVa}/C_{Ha}\}(1 + C_{Am}/J_{Am,HaVa} + C_{Ps}/J_{Ps,HaVa})] + v_{HaVa} f_{Po,Ma}/[1 + M_{Ha,HaVa}/C_{Ha}] \quad (9.11)$$

$U_{Ha,HaEx} = k_{HaEx} Q_{Ha}$ (9.12)

Differential eq: $dQ_{Ha}/dt = P_{Ha,WrHa} + P_{Ha,SrHa} + P_{Ha,SdHa} + P_{Ha,MaMd} + P_{Ha,LaHa} - U_{Ha,AmMa} - U_{Ha,PsMa} - U_{Ha,McMa} - U_{Ha,HaAs} - U_{Ha,HaVa} - U_{Ha,HaEx}$ (9.13)

Auxiliary eq: $v_{HaAs} = v^{*}_{HaAs} Q_{Ma}$ (9.14)

$v_{HaVa} = v^{*}_{HaVa} Q_{Ma}$ (9.15)

$k_{HaEx} = k_{FlEx}$ (9.16)

Hexose available to fibrolytic microbes, Q_{Hc} (mol)

Concentration: $C_{Hc} = Q_{Hc}/V_{Ru}$ (10.1)

Input: $P_{Hc,FdHc} = Y_{Hc,FdHc} U_{Fd,FdHc}$ (10.2)

Outputs: $U_{Hc,AmMc} = R_{Hc,AmMc} U_{Am,AmMc}$ (10.3)

$U_{Hc,PsMc} = R_{Hc,PsMc} U_{Ps,PsMc}$ (10.4)

$$U_{Hc,HcVa} = v_{HcVa}/[\{1 + M_{Hc,HcVa}/C_{Hc}\}(1 + C_{Am}/J_{Am,HcVa} + C_{Ps}/J_{Ps,HcVa})] \quad (10.5)$$

$U_{Hc,HcEx} = k_{HcEx} Q_{Hc}$ (10.6)

Differential eq: $dQ_{Ha}/dt = P_{Hc,FdHc} - U_{Hc,AmMc} - U_{Hc,PsMc} - U_{Hc,HcVa} - U_{Hc,HcEx}$ (10.7)

(continued)

Table A4 *(continued)*. *Mathematical statement of model*

Auxiliary eq: $v_{HcVa} = v^{*}_{HcVa} Q_{Mc}$ (10.8)

$k_{HcEx} = k_{FlEx}$ (10.9)

Microbial growth, passage and death

Amylolytic microbes pool, Q_{Ma} (g)

Concentration: $C_{Ma} = Q_{Ma}/V_{Ru}$ (11.1)

Inputs: $P_{Ma,AmMa} = Y_{Ma,AmMa} U_{Am,AmMa}$ (11.2)

$P_{Ma,PsMa} = Y_{Ma,PsMa} U_{Ps,PsMa}$ (11.3)

$P_{Ma,McMa} = Y_{Ma,McMa} U_{Mc,McMa}$ (11.4)

Outputs: $U_{Ma,MaMd} = k_{MaMd} Q_{Ma} f_{Po,Ma}$ (11.5)

$U_{Ma,MaEx} = k_{MaEx} Q_{Ma} [1 - f_{Po,Ma}]$ (11.6)

$U_{Ma,PoEx} = k_{PoEx} Q_{Ma} f_{Po,Ma}$ (11.7)

Differential eq: $dQ_{Ma}/dt = P_{Ma,AmMa} + P_{Ma,PsMa} + P_{Ma,McMa} - U_{Ma,MaMd} - U_{Ma,MaEx} - U_{Ma,PoEx}$ (11.8)

Auxiliary eq: $U_{Mc,McMa} = U_{Mc,McEg}/[1 + M_{Ha,McMa}/C_{Ha}]$ (11.9)

$k_{MaMd} = k_{MaEx} - k_{PoEx}$ (11.10)

$k_{MaEx} = k_{FlEx}$ (11.11)

$k_{PoEx} = 1/2\ k_{SoEx}$ (11.12)

Microbial storage polysaccharides pool, Q_{As} (g)

Concentration: $C_{As} = Q_{As}/V_{Ru}$ (12.1)

Input: $P_{As,HaAs} = Y_{As,HaAs} U_{Ha,HaAs}$ (12.2)

Outputs: $U_{As,MaMd} = k_{AsMd} Q_{As} f_{Po,Ma}$ (12.3)

$U_{As,AsEx} = k_{AsEx} Q_{As} [1 - f_{Po,Ma}]$ (12.4)

$U_{As,PoEx} = k_{PoEx} Q_{As} f_{Po,Ma}$ (12.5)

Differential eq: $dQ_{As}/dt = P_{As,HaAs} - U_{As,MaMd} - U_{As,AsEx} - U_{As,PoEx}$ (12.6)

Auxiliary eq: $k_{AsEx} = k_{FlEx}$ (12.7)

$k_{AsMd} = k_{MaEx} - k_{PoEx}$ (12.8)

Fibrolytic microbes pool, Q_{Mc} (g)

Concentration: $C_{Mc} = Q_{Mc}/V_{Ru}$ (13.1)

Inputs: $P_{Mc,AmMc} = Y_{Mc,AmMc} U_{Am,AmMc}$ (13.2)

$P_{Mc,PsMc} = Y_{Mc,PsMc} U_{Ps,PsMc}$ (13.3)

Outputs: $U_{Mc,McEg} = v_{McEg}/[1 + M_{Mc,McEg}/(C_{Mc} + C_{Ma}\{1-f_{Po,Ma}\})]$ (13.4)

$U_{Mc,McEx} = k_{McEx} Q_{Mc}$ (13.5)

Differential eq: $dQ_{Mc}/dt = P_{Mc,AmMc} + P_{Mc,PsMc} - U_{Mc,McEg} - U_{Mc,McEx}$ (13.6)

Auxiliary eq: $v_{McEg} = v^{*}_{McEg} Q_{Ma} f_{Po,Ma} C_{Mc}/[C_{Mc} + C_{Ma}\{1-f_{Po,Ma}\}]$ (13.7)

$k_{McEx} = k_{SoEx}$ (13.8)

(continued)

Table A4 *(continued). Mathematical statement of model*

Volatile fatty acids

Acetic acid pool, Q_{Ac} (mol)

Concentration: $C_{Ac} = Q_{Ac}/V_{Ru}$ (14.1)

Inputs: $P_{Ac,InAc} = Y_{Ac,InAc}D_{Ac}$ (14.2)

$P_{Ac,AmMa} = Y_{Ac,AmMa}U_{Hf,AmMa}$ (14.3)

$P_{Ac,AmMc} = Y_{Ac,AmMc}U_{Hf,AmMc}$ (14.4)

$P_{Ac,PsMa} = Y_{Ac,PsMa}U_{Hf,PsMa}$ (14.5)

$P_{Ac,PsMc} = Y_{Ac,PsMc}U_{Hf,PsMc}$ (14.6)

$P_{Ac,HaAs} = Y_{Ac,HaAs}U_{Hf,HaAs}$ (14.7)

$P_{Ac,HaVa} = Y_{Ac,HaVa}U_{Ha,HaVa}$ (14.8)

$P_{Ac,HcVa} = Y_{Ac,HcVa}U_{Hc,HcVa}$ (14.9)

$P_{MaAc,PsAm} = Y_{Ac,PsAm}U_{MaPs,PsAm}$ (14.10)

$P_{McAc,PsAm} = Y_{Ac,PsAm}U_{McPs,PsAm}$ (14.11)

$P_{Ac,McMa} = Y_{Ac,McMa}U_{Hf,McMa}$ (14.12)

$P_{Ac,McAm} = Y_{Ac,McAm}U_{Mc,McAm}$ (14.13)

Outputs: $U_{Ac,AcAb} = v_{AcAb}/[1 + M_{Ac,AcAb}/C_{Ac}]$ (14.14)

$U_{Ac,AcEx} = k_{AcEx}Q_{Ac}$ (14.15)

Differential eq: $dQ_{Ac}/dt = P_{Ac,InAc} + P_{Ac,AmMa} + P_{Ac,AmMc} + P_{Ac,PsMa} + P_{Ac,PsMc} + P_{Ac,HaAs} + P_{Ac,HaVa} + P_{Ac,HcVa} + P_{MaAc,PsAm} + P_{McAc,PsAm} + P_{Ac,McMa} + P_{Ac,McAm} - U_{Ac,AcAb} - U_{Ac,AcEx}$ (14.16)

Auxiliary eq: $U_{Hf,AmMa} = f_{Hf,AmMa}U_{Ha,AmMa}$ (14.17)

$U_{Hf,AmMc} = f_{Hf,AmMc}U_{Hc,AmMc}$ (14.18)

$U_{Hf,PsMa} = f_{Hf,PsMa}U_{Ha,PsMa}$ (14.19)

$U_{Hf,PsMc} = f_{Hf,PsMc}U_{Hc,PsMc}$ (14.20)

$U_{Hf,HaAs} = f_{Hf,HaAs}U_{Ha,HaAs}$ (14.21)

$U_{Hf,McMa} = f_{Hf,McMa}U_{Ha,McMa}$ (14.22)

$Y_{Ac,LaAc} = f_{Lc,Le}Y_{Ac,WrAc}$ (14.23)

$Y_{Ac,HaVa} = (Y_{Ac,WrAc}P_{Ha,WrHa} + Y_{Ac,LaAc}P_{Ha,LaHa} + Y_{Ac,StAc}(P_{Ha,SrHa} + P_{Ha,SdHa} + P_{Ha,MaMd}))/(P_{Ha,WrHa} + P_{Ha,LaHa} + P_{Ha,SrHa} + P_{Ha,SdHa} + P_{Ha,MaMd})$ (14.24)

$Y_{Ac,HcVa} = f_{Ce,Fd}Y_{Ac,CeAc} + (1 - f_{Ce,Fd})Y_{Ac,HeAc}$ (14.25)

$Y_{Ac,PsVa} = Y_{Ac,PsAc}$ (14.26)

$Y_{Ac,AmMa} = Y_{Ac,HaVa}$ (14.27)

$Y_{Ac,AmMc} = Y_{Ac,HcVa}$ (14.28)

$Y_{Ac,PsMa} = Y_{Ac,HaVa}$ (14.29)

$Y_{Ac,PsMc} = Y_{Ac,HcVa}$ (14.30)

$Y_{Ac,HaAs} = Y_{Ac,HaVa}$ (14.31)

(continued)

Table A4 *(continued). Mathematical statement of model*

$$Y_{Ac,McMa} = Y_{Ac,HcVa} \quad (14.32)$$

$$Y_{Ac,PsAm} = Y_{Ac,PsVa} \quad (14.33)$$

$$Y_{Ac,McAm} = Y_{Ac,PsVa} \quad (14.34)$$

$$v_{AcAb} = v^{*}_{AcAb} V_{Ru}^{0.75} / [1 + (pH/J_{pH,AcAb})^{\Phi pH,AcAb}] \quad (14.35)$$

$$k_{AcEx} = k_{FlEx} \quad (14.36)$$

Propionic acid pool, Q_{Pr} (mol)

Equations for this pool as for acetic acid pool but with Pr replacing Ac throughout

Butyric acid pool, Q_{Bu} (mol)

Equations for this pool as for acetic acid pool but with Bu replacing Ac throughout

Valeric acid pool, Q_{Vl} (mol)

Equations for this pool as for acetic acid pool but with Vl replacing Ac throughout

See Tables A1 and A2 for explanation of notation.

Chapter 2

SIMULATION OF NUTRIENT DIGESTION, ABSORPTION AND OUTFLOW IN THE RUMEN: MODEL EVALUATION

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SIMULATION OF NUTRIENT DIGESTION, ABSORPTION AND OUTFLOW IN THE RUMEN: MODEL EVALUATION

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A mathematical model of the rumen fermentation processes constructed to predict nutrient supply to the host animal was evaluated. Sensitivity analysis on high fibre, starch and protein diets indicated that the model responds appropriately to these types of diets and to changes in parameter values, and revealed that the model is sensitive to the availability of hexose for non-growth microbial processes and to the maximum storage rate of polysaccharides in amylolytic microbes, although sensitivity varied with diet composition. Of the parameters whose values were dependent on diet, the fraction of protozoa in the amylolytic microbial pool and the fluid and solid passage rates needed the most careful estimation. When model predictions of nutrient supply were compared with the experimental observations, those for duodenal flows of neutral detergent fibre and total non-ammonia nitrogen (NAN) and total volatile fatty acid rumen concentration were satisfactory for several feeding strategies. The partition of NAN flow into microbial and non-microbial NAN flow and the molar proportions of volatile fatty acid production and concentration were not predicted well. The representation of the complex interactions between rumen microbial populations and of their effects on the production of specific VFA merits further study for an improvement in the prediction of nutrient supply.

Rumen: Mathematical model: Ruminants: Microbial metabolism: Digestion

A number of mathematical representations of rumen metabolism have been developed with the aim of predicting the nutrients available for absorption from different diets (eg France *et al.* 1982; Baldwin *et al.* 1987). However, none of these representations can be considered to adequately predict nutrient availability in all dietary situations, including forage-based diets. In our companion paper (Dijkstra *et al.* 1992), we developed a model to predict the profile of nutrients available for absorption in diets containing forage. The model is depicted in the figures of the companion paper (Dijkstra *et al.* 1992) and a complete description of the model appears in the appendix of that paper. Preliminary evaluation of the model against some of the data used in its construction, to reproduce the results from an experiment in which grass hay-based diets were fed to steers at different nitrogen and intake levels, was adequate. This paper describes further evaluation of the model's behaviour and its simulations for other types of forages and supplements to assess the accuracy of model predictions and the adequacy of basic model concepts.

METHODS

Two kinds of evaluation are in order: first, sensitivity analysis to ensure that model outputs are appropriately sensitive to the given parameter values, and second, comparisons of the model predictions of the supply of nutrients to the host animal with the results from published experiments to examine the response of the model over a wider variety of diets than those used to parameterize the model.

Sensitivity and behaviourial analyses

The adequacy of some of the concepts and the quantitative information incorporated into the model and its overall stability were tested with the following sensitivity and behaviourial analyses. In line with the objective of the model, the sensitivity of the model was tested on silage-based diets, which are typical of cattle diets but also provide contrasting levels of the three major components of those diets, viz. fibre, amylolytic carbohydrate (starch and sugars) and protein. The chosen diets (intake 12 kg dry matter (DM)/d) were a high fibre diet (Diet S), consisting of grass silage (22.1 g nitrogen/kg DM, 546 g neutral detergent fibre (NDF)/kg DM and 20 g sugars/kg DM), a high starch diet (Diet SB), consisting of the same silage with 80% replaced by barley (18.8 g nitrogen/kg DM, 296 g NDF/kg DM and 454 g starch and sugars/kg DM) and a high protein diet (Diet SS), consisting of the same silage with 20% replaced by soya bean meal (33.9 g nitrogen/kg DM, 468 g NDF/kg DM and 40 g starch and sugars/kg DM). In simulations for these three diets fed to a 660 kg dairy cow, the selected parameters were set at +/- 50% and +/- 25% of the model value unless otherwise stated. For each parameter and diet combination, five steady-state solutions to the model equations were derived using the model value and the four sensitivity values.

The following parameters were selected for the sensitivity analyses either because there were insufficient data from the literature for an accurate estimation or because they were considered likely to have the greatest effect on some or all of the rumen processes.

Internal parameters

Because the Michaelis-Menten affinity parameters of energy utilization not related to growth ($M_{Ha,HaVa}$, $M_{Hc,HcVa}$; see Dijkstra *et al.* (1992) for coding conventions), and for hydrolysis of fibre, starch and protein ($M^*_{Fd,FdHc}$, $M^*_{Sd,SdHa}$ and $M^*_{Pd,PdPs}$) were estimated to tune the simulation outputs for those experiments, which were drawn upon to develop the model, it is important to examine the behaviour of the model over a range of values for these parameters. The maximum storage rate of polysaccharides in microbes (v^*_{HaAs}) and the maximum rate of absorption of ammonia (v^*_{AmAb}) were selected because of the possible effects on rumen digestion of nutrients and entry of endogenous urea into the rumen.

Input parameters

Input parameters included those that define the feed and the rumen characteristics particular to that diet. Changes in the fraction of protozoa in the amylolytic microbial pool ($f_{Po,Ma}$) would affect the amount of recycled microbial matter and provided an opportunity to examine the behaviour of the model with reference to defaunated animals. Fluid and solid passage rates (k_{FlEx} and k_{SoEx}) and pH and rumen volume (V_{Ru}) were selected because they could have profound effects on rumen fermentation characteristics.

Comparison of model predictions with experimental results

Data on nutrient supply from several experiments, other than those drawn upon to develop the model, were used for validation. The choice of experiments to challenge the model was based on adequate information to allow simulation and on amount of data on nutrient supply reported, where these had either been measured in animals offered feed at frequent intervals or were average values obtained from data on discontinuously fed animals, to correspond with the steady-state solutions of the model. The choice was restricted to forage-based diets with a wide variation in intake level, roughage to concentrate ratio, and fibre, starch, sugars, nitrogen and lipid levels, in line with the model. However, although outflow of these components and rumen concentrations have been measured in several experiments, it should be noted that, to date, data sets on cattle providing a complete profile of nutrients for absorption have not been reported; hence a full comparison of predicted and observed values cannot be made. Where digestion turnover time had not been measured, analysis of the feed was insufficient or other parameters required for input to the model were missing, the guidelines and sources of data recommended in the companion paper (Dijkstra *et al.* 1992) or values from similar experiments were used. Where NDF faecal flow or total tract NDF digestibility was given but not rumen NDF duodenal flow or digestibility, the NDF duodenal flow was estimated as the NDF faecal flow divided by 0.85, based on studies of Tamminga (1981). The NDF duodenal flow was estimated in this manner for the experiments reported by Robinson *et al.* (1987) and Horner *et al.* (1988).

The assessment of error of predicted values relative to experimental values was made on the calculation of the mean square prediction error (MSPE):

$$MSPE = \Sigma(O_i - P_i)^2/n$$

where $i = 1, 2, \ldots n$; n is the number of experimental observations; and O_i and P_i are the observed and predicted values (Bibby & Toutenburg, 1977). The root MSPE is a measure in the same units as the output and is presented in the tables as a percentage of the observed mean. The MSPE was decomposed into the overall bias of prediction, deviation of the regression slope from one, and the disturbance proportion (Bibby & Toutenburg, 1977). The experiments used for comparison fall into

Table 1. *Intake analysis of diets used for model validation*

Study	Treatment	Intake			
		DM	NDF	Starch and sugars	N
		kg/d		*g/d*	
Robinson *et al.* (1987)	low intake	5.3	2268	1227	138
	low medium intake	9.2	3785	2147	249
	medium intake	13.1	5276	3066	348
	high medium intake	17.1	6774	3986	449
	high intake	21.0	8295	4906	550
	8% starch	13.1	6313	1943	339
	14% starch	13.1	5683	2507	344
	20% starch	13.1	5211	3071	344
	26% starch	13.1	4817	3609	350
	32% starch	13.1	4383	4174	357
Robinson *et al.* (1985)	low intake	6.4	2746	1608	164
	low medium intake	11.3	4826	2826	263
	high medium intake	15.5	6650	3894	377
	high intake	18.5	7937	4648	448
Horner *et al.* (1988)	0% cottonseed	8.1	2937	3228	196
	15% cottonseed	8.4	3256	2886	204
McCarthy *et al.* (1989)[1]	CF	23.3	8015	10981	541
	CS	24.2	7308	11788	581
	BF	20.5	7298	9061	480
	BS	20.9	6793	9526	497
Robinson & Kennelly (1990)[2]	RDP	19.4	7868	4457	568
	UDP	18.3	7700	4061	540
Beever *et al.* (1990)	control	4.1	2233	82	90
	low fishmeal substitution	4.1	2132	78	108
	high fishmeal substitution	4.2	1926	71	143
Firkins *et al.* (1984)[3]	U	6.8	3140	2955	144
	WDG	6.8	3899	1831	163
	DDG	6.8	3876	1831	170
	WCGF	6.8	3447	2033	165
	DCGF	6.8	3433	2033	161
Rogers & Davis (1982a)	medium concentrate	5.2	1433	2266	91
Rogers & Davis (1982b)	high concentrate	7.7	1771	4151	139
	low concentrate	9.0	3807	1530	200
Lebzien (1980)	low roughage	12.0	4158	4322	346
	high roughage	14.2	7076	2934	327

DM, dry matter; N, nitrogen; NDF, neutral detergent fibre.

[1] Forage diets supplemented with corn and fish meal (CF), corn and soya bean meal (CS), barley and fish meal (BF) or barley and soya bean meal (BS).

[2] Concentrate containing rapidly (RDP) or slowly (UDP) degradable protein source.

[3] Basal diet supplemented with urea (U), wet (WDG) or dry (DDG) distillers grains, or wet (WCGF) or dry (DCGF) corn gluten feed.

three groups: experiments with high energy supplements, experiments with nitrogenous supplements and experiments where volatile fatty acid (VFA) production was measured. The animals involved included dry cows, lactating cows and fast growing steers. Description of the experiments follow and intake analyses are listed in Table 1.

Experiments with high energy supplements

The effect of feed intake level and of starch concentration was reported by Robinson *et al.* (1986 and 1987). They fed Dutch Friesian lactating dairy cows diets of 1/3 hay and 2/3 pelleted concentrates, containing five graded levels of starch between 8 to 32%, and at five intake levels between 5.3 to 21.0 kg DM/d (Table 1).

Robinson *et al.* (1985) reported the effect of intake levels (6.4 to 18.5 kg DM/d) on digestion and bacterial yield for Holstein dairy cows fed a 65% hay, 35% concentrate (corn-soya bean meal mix) diet. The diet was offered two, four or eight times daily. Diaminopimelic acid (DAPA) was used as microbial marker. Because DAPA is generally assumed to measure bacterial biomass only, model predictions of "microbial" non-ammonia nitrogen (NAN) outflow for this experiment excluded the predicted protozoal flow.

When whole cottonseed was used as a source of dietary fat to supplement a 40% corn-soya bean meal, 60% hay diet offered to non-lactating Holstein heifers (Horner *et al.* 1988), the effect on ruminal digestion was minimal (treatments: 0% and 15% whole cottonseed, Table 1).

To investigate the effect of source of carbohydrate and protein on ruminal fermentation and flow of nutrients to the small intestine, four lactating Holstein dairy cows were offered ad libitum access to complete diets containing shelled corn or barley and fish meal or soya bean meal (McCarthy *et al.* 1989) (treatments: CF, corn and fish meal; CS, corn and soya bean meal; BF, barley and fish meal; BS, barley and soya bean meal; Table 1).

Experiments with nitrogenous supplements

Robinson & Kennelly (1990) investigated the effect of including a rapidly or slowly degradable protein source in the concentrate on duodenal digesta flow and rumen fermentation in lactating dairy cows fed 50% forage, 50% concentrate diets. The forage consisted of whole crop oat silage, second cut alfalfa silage and hay. The concentrate provided protein from a rapidly degradable source (canola meal: treatment RDP) or from a slowly degradable source (distillers grains and fish meal: treatment UDP, Table 1). As with the Robinson *et al.* (1985) experiment, DAPA was used as microbial marker.

The effects of supplementation of grass silage diets with fish meal on rumen digestion of organic matter and nitrogen was investigated by Beever *et al.* (1990). Young growing cattle were fed a good quality lactic acid perennial ryegrass silage in three diets: C: silage only; FM1, silage + fish meal at 50 g/kg total DM; and FM2,

Table 2. *Model response and extreme point responses of rumen parameters in sensitivity analysis*

Parameter /diet	Model value	Rumen ammonia	Duodenal flow				
			Total NAN	Microbial NAN	Total fibre	Total starch + sugars	Microbial polysaccharide
		mM			*g/d*		
Model response							
S		5.3	277.3	209.7	1648.2	334.3	35.9
SB		2.6	271.7	224.4	1431.4	783.6	664.4
SS		24.0	308.6	194.3	1495.6	232.3	58.7
$M_{Hx,HxVa}$[1]	55 mM	Range tested 27.5 - 82.5 mM					
S		6.7-4.8	262.6-283.9	190.7-217.9	1684.5-1634.9	322.9-340.4	28.5-39.9
SB		4.0-2.0	254.0-280.3	201.6-235.0	1467.6-1415.7	686.9-839.8	565.8-720.0
SS		24.7-23.8	305.7-309.6	187.0-196.7	1504.3-1493.9	223.3-236.8	49.0-63.3
v^*_{HaAs}	53 mmol/(g Ma·d)	Range tested 26.5 - 79.5 mmol/(g Ma·d)					
S		5.5-5.2	275.6-278.8	207.6-211.5	1654.5-1642.9	322.1-345.1	19.4-50.1
SB		2.0-3.1	280.2-264.4	234.3-215.7	1476.5-1396.3	488.2-1028.7	369.9-907.7
SS		24.3-23.8	307.4-309.8	191.9-196.5	1505.1-1487.6	207.2-253.8	31.8-81.6
$f_{Po,Ma}$		Range tested 0.0 - 1.0					
S	0.70	2.7-6.6	313.6-263.6	251.1-193.6	1583.7-1676.7	345.9-342.2	70.2-16.8
SB	0.20	0.4-25.4	307.4-140.5	268.6-43.4	1138.3-3561.6	1029.0-532.1	864.4-317.2
SS	0.58	15.8-28.7	353.4-288.0	258.8-158.0	1398.3-1570.0	250.2-227.0	96.2-24.6
k_{FlEx}	/d	Range tested 0.9 - 2.7/d					
S	2.00	13.6-3.7	228.0-294.6	164.6-224.6	1775.3-1622.8	107.6-700.9	60.6-18.8
SB	1.46	3.9-1.6	258.1-283.7	223.0-214.2	1629.6-1293.0	726.0-1163.6	674.6-675.4
SS	1.87	60.3-16.2	259.0-332.2	155.8-209.0	1612.9-1469.2	118.1-458.6	81.1-42.6
k_{SoEx}	/d	Range tested 0.4 - 1.2/d					
S	0.77	7.2-4.6	257.0-286.4	207.1-193.2	1330.7-2270.1	304.5-366.4	30.8-41.5
SB	0.80	4.2-2.2	252.1-276.5	215.6-217.2	1054.4-2090.8	741.1-825.9	646.5-683.6
SS	0.78	34.5-16.2	256.7-350.7	187.3-179.7	1191.6-2055.5	215.2-252.0	52.3-65.8

See Appendix of Dijkstra *et al.* (1992) for notation of parameters. Sensitivity diets: S, silage only; SB, same silage with 80% dry matter replaced by barley; SS, same silage with 20% dry matter replaced with soya bean meal; NAN, non-ammonia nitrogen.
[1] Hx indicates a simultaneous test on $M_{Ha,HaVa}$ and $M_{Hc,HcVa}$, because they are set equal in the model.

silage + fish meal at 150 g/kg total DM. The mean intake was 4.1 kg DM/d with a nitrogen intake ranging from 90 to 143 g nitrogen/d over the three diets.

The effects of urea, wet and dry distillers grains and corn gluten feed on fermentation and digesta flows in Angus-Hereford steers fed a basal diet of hay, corn and corn cobs were examined by Firkins *et al.* (1984). Intake was 6.8 kg DM/d for all treatments, with urea supplement: U, with wet distillers grains: WDG, with dry distillers grains: DDG; with wet corn gluten feed: WCGF and with dry corn gluten feed: DCGF (Table 1).

Experiments with measured VFA production

The agreement between VFA molar proportions in rumen fluid and VFA proportions produced have been questioned (Sutton, 1985). Thus, experiments in which the production of the major VFA (acetic, propionic, and butyric acids) were measured in the rumen by isotope-dilution techniques are considered to be of interest for validation. In an experiment of Rogers & Davis (1982a), young steers were fed 5.2 kg DM/d of a corn silage-based 50% concentrate, 50% roughage diet (MC). In another experiment reported by these authors (Rogers & Davis, 1982b), heavier steers were fed either 7.7 kg DM/d of a 75% concentrate, 25% roughage diet (HC) or 9.0 kg DM/d of an 18% concentrate, 82% roughage diet (LC).

Another experiment concerned with the measurement of VFA production was conducted by Lebzien (1980), in which two diets, a 55% concentrate, 45% roughage diet (LR) and a 13% concentrate, 87% roughage diet (HR), were fed to lactating black and white dairy cattle (Table 1).

RESULTS AND DISCUSSION

Sensitivity and behaviourial analyses

The model predictions of ammonia concentration and the duodenal flows of nitrogen, NDF, and starch and sugars for the three sensitivity diets and the responses to the extreme points of the sensitivity ranges of the five parameters to which the model proved most sensitive are shown in Table 2. The response to the other selected parameters is described in the text.

The behaviour of the model for the 3 sensitivity diets was as expected. The high nitrogen diet (SS) had the highest ammonia concentration, and the high starch and low nitrogen diet (SB) the lowest ammonia concentration, which is as would be expected from the available nitrogen to energy ratios of the three diets. The duodenal NAN flow increased with the nitrogen content of the diet, whereas the microbial NAN decreased (Table 2). In general, increasing the concentrate (starch) proportion of the diet will decrease the digestion of NDF in the rumen (Erdman, 1988). The simulations agreed with this. The NDF digestion with Diet SB (59.7%) was much lower than with the unsupplemented diet (74.8%). The duodenal flow of polysaccharides was 2% of total microbial mass for Diet S and 26% for Diet SB. This is in line with the literature

(McAllan & Smith, 1977) and in contrast to previous models in which the microbial composition was held constant. The properties of the diets were also reflected in the simulated production and molar proportions of VFA. Diet SB showed the highest VFA production rate (Diet SB, 59.1 mol/d, Diet S, 52.1 mol/d; and Diet SS, 50.6 mol/d). As a result of the stoichiometric parameters applied, molar proportions of propionic acid of Diet SB as compared with Diet S were greater (27.1 vs. 13.0%) and those of acetic acid were less (55.9 vs. 72.6%), which is generally accepted from results of experiments with roughage diets supplemented with energy-rich ingredients (Sutton, 1985). Because valeric acid is a major end product of amino acid fermentation, Diet SS showed a higher molar proportion of this acid compared with Diet S (8.8 vs. 6.1%). These simulations indicate that the supply of glucogenic precursors (starch and sugars escaping fermentation, microbial polysaccharides and glucogenic amino acids washed out and propionic acid absorbed or washed out) is much higher with Diet SB than with Diet S. This could give rise to differences in efficiency of energy utilization in ruminants observed or in various simulated diets (Gill *et al.* 1984). In conclusion, results from changes in diet composition are in line with literature, and properties of each diet are reflected in the simulated nutrients available for absorption.

Internal parameters

An increase in $M_{Ha,HaVa}$ and $M_{Hc,HcVa}$ means that less hexose is utilized for processes not related to growth, and hence microbial production and flow into duodenum are increased. This leads to lower ammonia concentrations, especially with Diet SB, because more ammonia is used for growth. As the availability of hexose for growth functions was increased, more hexose was utilized to form microbial storage polysaccharides, in line with experimental findings (Mulder, 1988). Although these internal productions were greatly affected, the effect on the duodenal flows of fibre (1% of model response for Diet SS, 4% for Diet SB), total NAN (1%, 10%) and starches and sugars (6%, 20%) and VFA production (4%, 8%) was much less and decreased with increasing nitrogen content of the diet (Table 2).

Increase of $M^{*}_{Pd,PdPs}$ decreased hydrolysis of protein in rumen. This decreased the concentration of both ammonia (15, 22 and 17% of model response for Diets S, SB and SS, respectively) and soluble protein and hence microbial growth (0.6, 0.7 and 0.3%). With decreasing availability of nitrogen, particularly with Diet SB, microbial growth was more depressed than fibre or starch digestion as a result of energetic uncoupling (decrease in microbial growth without concomitant decrease in organic matter digestion, Hespell & Bryant, 1979). Also, the slight increase in microbial storage polysaccharides (0.7% of model response on Diet SB) simulated when increasing $M^{*}_{Pd,PdPs}$ agrees with Hespell & Bryant (1979), who stated that nutrient limitations (usually nitrogen limitation) will give rise to a relatively higher rate of formation of microbial polysaccharides.

The duodenal flow of potentially degradable fibre was very sensitive to an increase

in $M^*_{Fd,FdHc}$. There was over all diets a mean increase of 100% of the mean model response of 439 g/d over the sensitivity range of 0.166 to 0.498 mol/l. However, the decrease in growth of fibrolytic microbes (maximum 22% of model response for Diet SB), microbial NAN duodenal flow (maximum 9% for Diet SS) and VFA production (maximum 8% for Diet SB) was less marked.

Increasing $M^*_{Sd,SdHa}$ would only affect the outputs for diets containing insoluble starch, but even with Diet SB, there was only a slight increase in the duodenal flow of total starch and sugars (5% model response of 784 g/d for Diet SB over sensitivity range for $M^*_{Sd,SdHa}$ of 0.208 to 0.624 mol/l). Because more than 60% of barley starch is soluble and the insoluble part has a low digestion turnover time (0.17 d), changes of the value of $M^*_{Sd,SdHa}$ would not have a large effect. In simulations in which corn starch replaced barley starch (results not shown), there was a considerably greater response, because the proportion of soluble starch and the degradation rate of the insoluble part of corn starch are lower.

As with the previous parameter, the effect of changes in the maximum storage rate of polysaccharides in microbes (v^*_{HaAs}) depended to a great extent on the diet composition (Table 2). With any diet the amount of amylolytic hexose directed towards formation of storage polysaccharides was increased, but the greater the energy concentration of the diet, the greater the sensitivity to this parameter. In Diet SB, less of the amylolytic hexose was available for microbial polysaccharide-free growth, and amylolytic microbial production was decreased. The glucogenic supply to the duodenum increased substantially however, due to the increased storage polysaccharide flow. The VFA production was reduced (5% of model response for Diet SB, 1% for Diets S and SS), because the formation of amylolytic storage polysaccharides does not require much energy (Hespell & Bryant, 1979).

Increasing the maximum rate of absorption of ammonia (v^*_{AmAb}) greatly decreased the concentration of ammonia (a maximum of 62% of model response of 24 mM for Diet SS) through an increase in absorption rate (maximum 79% of model response of 5.04 mol/d), but the ammonia concentration was still too high to affect microbial growth to a significant extent (Hespell & Bryant, 1979). However, the fibrolytic microbes were slightly more sensitive to a decrease in ammonia concentration (on average 1.9 vs. 1.2% of the model response).

Input parameters

An increase in the fraction of protozoa in the amylolytic microbial pool ($f_{Po,Ma}$) and consequently an increase in the amount of recycled microbial matter had a profound effect on the type of bacteria present (Figure 1; Table 2). The number of fibrolytic bacteria decreased as a result of protozoal predation, and this change was accompanied by a nonlinear increase in number of amylolytic microbes with Diet SB. These changes resulted in an overall decrease of total microbial mass in the rumen. Both total NAN and microbial NAN outflow decreased, whereas ammonia concentration and absorption increased, relatively more so with low nitrogen diets.

As the ratio of fibrolytic to amylolytic microbes decreased, the amount of microbial storage polysaccharides per unit microbial matter present increased.

Variation in $f_{Po,Ma}$ provides an opportunity to examine the behaviour of the model with reference to defaunated animals (Table 2). In a review, Jouany *et al.* (1988) discussed the complex interactions between protozoa and amylolytic and fibrolytic bacteria in relation to dietary and rumen fluid properties. Defaunation was stated to lower fibrolytic activity with a diet rich in cell wall carbohydrates, but with an easily fermentable carbohydrate diet, fed in restricted amounts, the opposite was observed. In contrast, the model predicted that as a result of defaunation with every diet, fibrolytic bacterial numbers increased, since the direct engulfment effect is higher than an effect of changes in ammonia concentration. Only when pH values are decreased are reduced fibrolytic numbers and reduced fibre digestion predicted. Such a reduction might be expected with defaunated animals in the absence of the moderation of the post prandial drop in pH by protozoal engulfment of starch particles and their subsequent slower metabolism. The predicted increase in total NAN and microbial NAN outflow and marked decrease in ammonia concentration is in line with observations on defaunated animals. However, both the increased feed protein and NDF degradation predicted are in contrast with most observations and suggest that metabolism of protozoa differs in more respects from metabolism of bacteria than has been modeled. The simulated changes on VFA molar proportions varied with the diet, in line with the review of Jouany *et al.* (1988). With each diet, molar proportions of acetic acid increased and those valeric acid decreased. With Diet SB, both propionic acid and butyric acid decreased, but on diet S and SS, propionic acid decreased and butyric acid increased as $f_{Po,Ma}$ decreased. These results clearly indicate that the supply of nutrients available for absorption varies with the diet fed, and this might affect the resultant animal performance to a different extent. Considering the importance of $f_{Po,Ma}$, this parameter should be accurately estimated for a range of diets. Unfortunately, the restricted data available do not allow such an estimation. Equally, although major aspects of protozoal metabolism have been modeled satisfactorily, the results indicate the need for a more detailed representation of protozoal metabolism. However, both the level of organization of predictive rumen models and the lack of knowledge and data of complex microbial interactions hamper a more detailed representation (Dijkstra *et al.* 1990).

Changes in the fluid passage rate (k_{FlEx}) had profound effects on rumen fermentation characteristics and were generally in line with literature (review Owens & Goetsch, 1986). A range of 0.9 to 2.7/d was chosen for the test (Table 2). An increase in k_{FlEx} did particularly affect the production rate of amylolytic microbes (with a maximum range of 80% model prediction for Diet S), and this change was accompanied by a marked increase in outflow of starch and sugars. Total NAN duodenal flow was increased except with Diet SB, for which the outflow of fibrolytic bacteria increased but the amylolytic bacterial outflow decreased. The more rapid flow of liquid decreased the ammonia and VFA concentrations (with maximum ranges

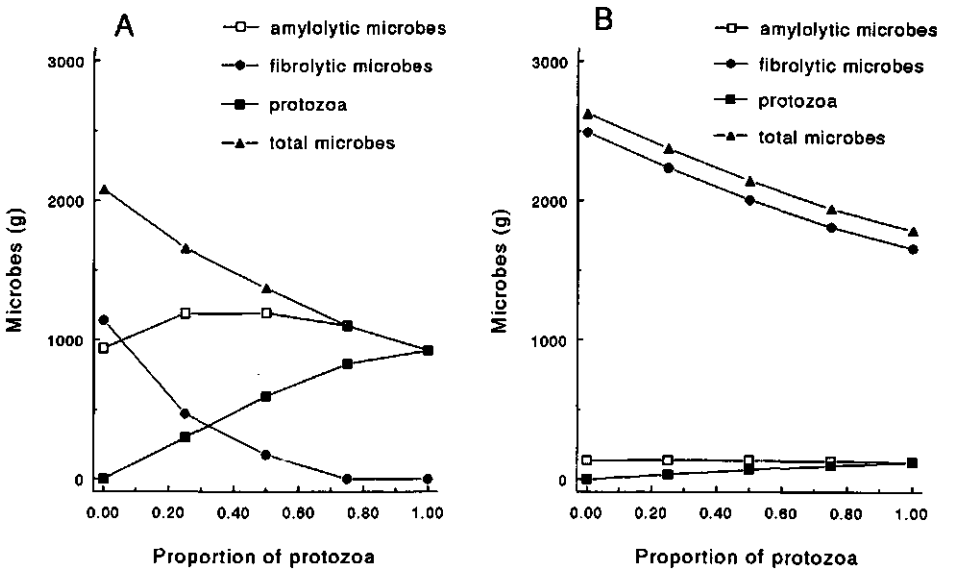

Figure 1. The sensitivity of the total microbial (▲), amylolytic microbial (□), fibrolytic microbial (●) and protozoal (■) pools to the fraction of protozoa in the amylolytic microbial pool ($f_{Po,Ma}$; steady-state solutions derived for values of $f_{Po,Ma}$ of 0, 0.25, 0.5, 0.75 and 1) for high starch diet (diet SB; graph A) and high protein diet (diet SS; graph B).

of 186 and 54% model prediction for Diet S) and absorption rates (47%). The microbial growth efficiency increased, as can be seen from the minor changes in microbial NAN outflow in relation to changes in ruminal digestion of the main energy-yielding components (fibre, starch and sugars), in line with increases in microbial efficiency generally observed (Owens & Goetsch, 1986).

In contrast with changes in k_{FIEx}, an increase in the solid passage rate (k_{SoEx}) particularly affected fibrolytic microbes and fibre digestion. The effect on the microbial NAN outflow was nonlinear as the amylolytic bacteria duodenal flow was increased, but at a slower rate than the fibrolytic bacteria outflow, which was directly affected by the change in solid passage rate (Table 2). Ammonia concentration was decreased because less feed protein was degraded (total NAN was increased while microbial NAN was decreased). The VFA concentration decreased because less fibre was digested and a higher portion of the digested fibre was directed towards the growth of microbial cells than towards VFA. Again, the model is very sensitive to this parameter, but the behaviour of the model to changes are in accordance with the literature (Owens & Goetsch, 1986).

Over the pH range chosen (5.6 to 7.6) to keep the other related parameters

realistic (viz. time below critical pH and minimum pH, Dijkstra *et al.* 1992), only the increase in the absorption of ammonia (average 106% of model response) and hence the decrease in concentration of ammonia (67-105%) with increasing pH were sensitive outputs. As in the test on v^*_{AmAb}, ammonia concentration has to be reduced to rather low levels before other ruminal processes are affected. The absorption of VFA was less affected (40% on average), but because most VFA not absorbed in the rumen will be available for postruminal absorption, the effect on total supply of energy to the animal will not be greatly affected. The hydrolysis of fibre was not affected because the inhibition period of fibre digestion, even with Diet SB, is short.

Increase in V_{Ru} directly affected the concentrations of VFA (at + 50% of model value, decreases of 146, 134 and 145% of model response for Diets S, SB and SS, respectively) and ammonia (74, 45 and 55%). Fibre digestion decreased (8, 15 and 9%) and the duodenal flow of starch and sugars increased (123, 41 and 85%). There was an increase in total (15, 11 and 21%), microbial and non-microbial NAN outflows. Because the effect of increasing rumen volume has not been investigated, no comparison can be made with results from literature.

From these tests, the model is shown to respond accurately to differences in diet composition and to be sensitive to the availability of hexose for microbial non-growth processes, and to the maximum storage rate of amylolytic polysaccharides. For diet-dependent parameters, the fraction of protozoa in the amylolytic microbial pool and the solid and fluid passage rates need the most careful estimation.

Comparison of model predictions with experimental observations

The comparisons of model predictions of rumen outflows with observed experimental values are examined under the main topics of fibre digestion, digestion and absorption of nitrogen, lipid representation and VFA concentration and production, on the overall data (Figures 2-4) and within experiments (Tables 3-5). Generally, differences between simulated and observed values should not be considered entirely due to inadequacy of the model, because inadequacies of input data describing the diets or errors in experimental measurements could also contribute to these differences.

Fibre digestibility

Predicted NDF outflows were in general quite close to observed values within and among experiments (Figure 2) with a root MSPE of 367 g, i.e., 13% of observed mean. The contribution of the random variation about the regression line component towards the MSPE was 84%. Although the amount of NDF washed out from the rumen generally is not a major contributor to the total available nutrient supply, since post ruminal NDF digestibility is low (Robinson *et al.* 1987), correct predictions of NDF outflow indicate that the fibrolytic microbial mechanism has been represented well. In this experiment, the prediction of the effect of increasing intake on rumen NDF outflow was closer to the observed value (13% of observed mean) than the prediction of the effect of increasing starch level (18%). Although observed NDF

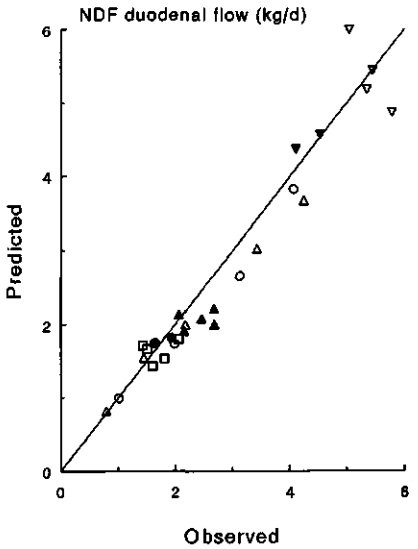

Figure 2. Comparison of observed and predicted values of neutral detergent fibre (NDF) rumen outflow. Code for experiments: □ Firkins *et al.* (1984); O Robinson *et al.* (1985); ● Horner *et al.* (1988); Robinson *et al.* (1987) - Δ intake level and ▲ starch level; ∇ McCarthy *et al.* (1989); ▼ Robinson & Kennelly (1990); ◇ Rogers & Davis (1982a and 1982b); ♦ Lebzien (1980); + Beever *et al.* (1990). The line of unit slope represents the line of accurate prediction.

outflows decreased with an increasing starch content of the diet, there was little change in model predictions. In line with results of the sensitivity analysis, this suggests that recycling of microbial matter, which in the present model is included by engulfment of fibrolytic microbes and death of protozoa only, needs to be represented in more detail for diets differing widely in starch or fibre content. A reduction in NDF digestibility with an increase in intake level was observed by Robinson *et al.* (1985) as well, and this reduction was simulated satisfactorily.

Both model predictions and observed values indicated less NDF digestion (43 and 34%) with the added urea treatment (Firkins *et al.* 1984). However, the observed values showed that NDF digestion was greatest with the added distillers grains treatment (distillers grains: 61 and 63%; corn gluten feed 47 and 54%), whereas the model predictions indicated little difference between the effects of distillers grains and corn gluten feed on NDF digestion (57, 56, 58 and 55%). This may be due to unknown differences in the feeds. In the experiment conducted by Horner *et al.* (1988), observed NDF digestion was reduced by supplementation with whole cottonseed (44% with 0% whole cottonseed and 41% on 15% whole cottonseed),

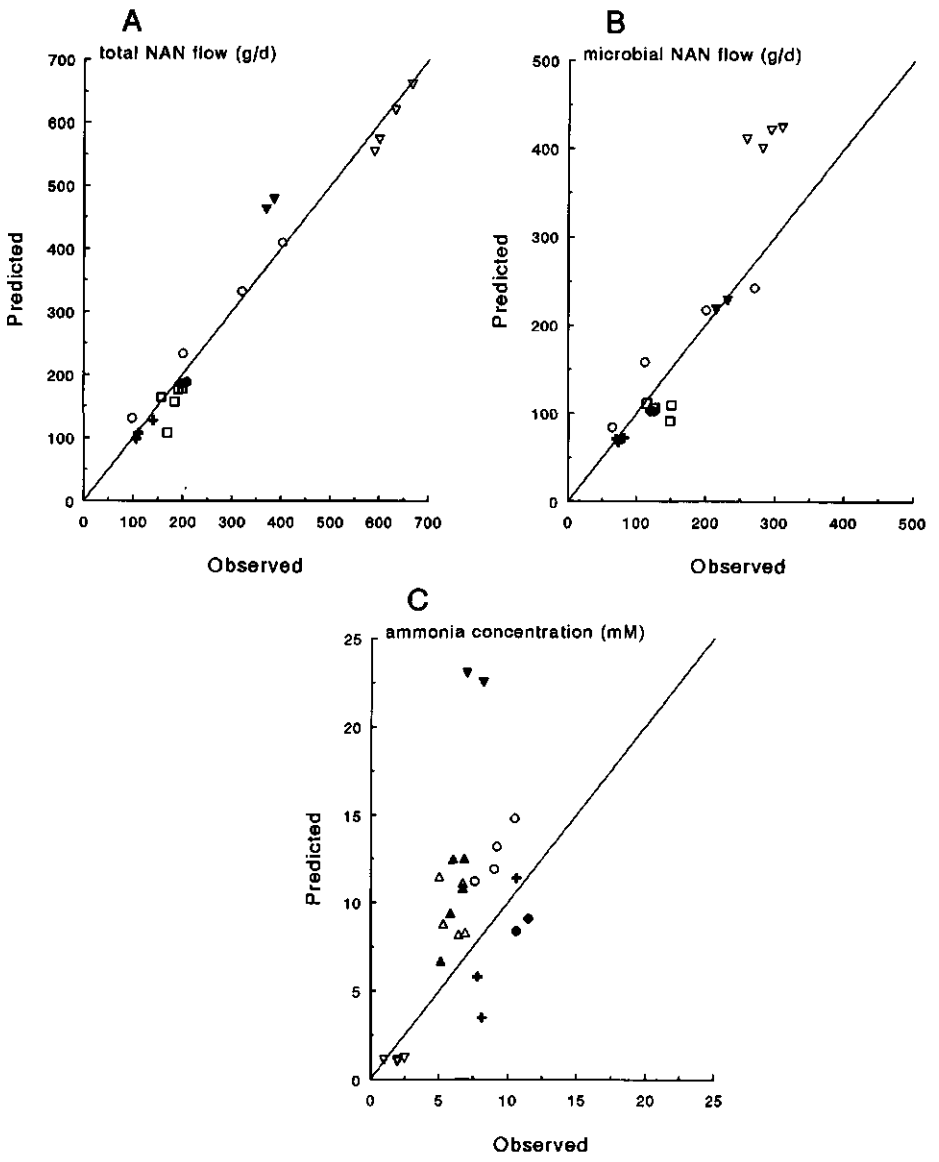

Figure 3. Comparison of observed and predicted values of total non-ammonia nitrogen (NAN) rumen outflow (graph A), microbial NAN rumen outflow (B) and rumen ammonia concentration (C). See footnote to Figure 2 for key to experiment symbols.

and model predictions were close to observed values. Based on several experiments examining the effects of lipid supplementation on rumen fermentation characteristics (survey by Van Nevel & Demeyer, 1988), a depressive effect on NDF digestion was expected. However, this effect was not included in the model and might account for these small differences. It should be noted that duodenal NDF flow was estimated from faecal NDF flow (see Methods), which could contribute to differences between observed and predicted values. The observed NDF digestion for the barley and soya bean meal diet reported by McCarthy *et al.* (1989) was considered extremely low (11% with BS vs. 32, 28 and 24% with CF, CS and BF, respectively). The value predicted by the model (28%) might be considered more likely. Predicted NDF rumen digestion coefficients were close to values observed by Robinson & Kennelly (1990). Both experiments had low rumen pH values (mean pH of 5.8). A reduction in plant cell wall digestion due to low pH values has been reported in vitro and in vivo (Erdman, 1988). These model simulations indicate that the depressing effect of pH on fibre digestion, simulated by a critical pH value and time below this value, has been represented adequately.

Digestibility and absorption of nitrogen

Predicted NAN outflow from the rumen is plotted against observed NAN outflow in Figure 3. The model seemed to predict total NAN outflow well for diets supplemented with energy or protein at several intake levels (root MSPE of 34 g, i.e., 13% of observed mean and 94% of MSPE attributed to the disturbance proportion), but when partitioned into microbial and non-microbial fractions the predictions were not so close (37 and 58%, respectively). The contribution of the deviation of the slope to unity proportion was 69% for microbial NAN-outflow, indicating proportional bias due to inadequate representation of relationships. However, the coefficient of variation

Table 3. *Comparison of experimental observations (obs) with model predictions (pred) of rumen bacterial nitrogen (N) for cows fed diets at different intake levels and containing different levels of starch*

Intake level (kg dry matter/d)	Rumen bacterial N		Starch level	Rumen bacterial N	
	obs	pred		obs	pred
	g			*g*	
5.3	87	85	8%	134	193
9.2	128	113	14%	142	179
13.1	157	162	20%	127	145
17.1	168	172	26%	169	147
21.0	183	241	32%	151	121
	%			%	
Error %	18.6		Error %	25.0	

Observations from Robinson *et al.* (1986 and 1987).

of observed microbial and non-microbial NAN outflow from the rumen is generally much larger than the coefficients of variation of total NAN flow (Oldham & Tamminga, 1980). Differences in microbial markers used, techniques of isolating rumen microbial DM and differences in times of sampling all contribute to the larger coefficient associated with microbial NAN flow and indirectly to that of the non-microbial NAN flow, because it is calculated as the difference between the other two flows. Thus, evaluation of the major determinant of rumen fermentation (the microbial metabolic activity) is limited and will be restricted to qualitative evaluation only. In the experiment of Robinson *et al.* (1987), the increase in rumen concentration of bacterial nitrogen was well predicted at the four lower levels of intake, but there was an overprediction at the highest level, with an overall error of 19% of the observed mean (Table 3). The steadily decreasing bacterial nitrogen content predicted with increasing starch levels, with an overprediction of 25% of the observed mean, was in contradiction to experimental values, in which there was no such pattern. Again, this might be related to an insufficient representation of microbial turnover as explained before.

In the study of Robinson & Kennelly (1990), the duodenal flows of NAN and starch were overpredicted (Figure 3). This might be related to the fact that the marker used was chromium, which is a particulate marker. Using CoEDTA, DM flow was 49% higher, as stated by Robinson & Kennelly (1990). Because 76 and 73% of feed starch and 49 and 43% of feed nitrogen is soluble for RDP and UDP respectively, the duodenal flows of these two moieties could be up to 150% higher. The predicted values were roughly 125% higher. Feed NAN was underestimated by the model and microbial flow overestimated for all treatments in the experiment of McCarthy *et al.* (1989) experiment. The authors commented that a larger proportion of dietary NAN passed to the duodenum than in other trials. Their conclusion, that microbial NAN was increased when barley replaced corn and that corn nitrogen is less degradable than barley nitrogen, was supported qualitatively if not quantitatively by the model results.

The prediction for microbial NAN outflow for urea treatment in Firkins *et al.* (1984) was markedly lower than the underprediction for the other four treatments (Table 4). The authors remarked that because the observed microbial NAN duodenal flow equalled that of the high protein diet and NDF digestion was only moderate (34%), this result seemed unlikely. The overall underprediction of microbial NAN in this experiment and in that of Horner *et al.* (1988) may be related to the fact that nucleic acids were used as the microbial marker, because results obtained with nucleic acids are generally higher than for other markers (Harrison & McAllan, 1980). Both in the experiment of Firkins *et al.* (1984) and that of McCarthy *et al.* (1989), observed duodenal nitrogen flow was higher than nitrogen intake with the feed (12 and 19% higher, respectively). Because predicted total NAN outflow was satisfactory, these results indicate that recycling of protein and urea by saliva and absorption through the rumen wall has been represented satisfactorily for the level of aggregation adopted.

Table 4. *Comparison of experimental observations (obs) with model predictions (pred) of non-ammonia nitrogen (NAN) duodenal flow and ammonia concentration for steers fed diets supplemented with different protein sources*

Treatment[1]	Duodenal flow				Ammonia concentration	
	Total NAN		Microbial NAN			
	obs	pred	obs	pred	obs	pred
	g/d				*mM*	
U	169	108	148	91	-	13.2
WDG	192	176	127	106	-	5.3
DDG	200	178	125	106	-	6.2
WCGF	157	164	115	111	-	7.2
DCGF	184	157	151	109	-	7.8
	%		%			
Error %	17.9		25.6			

Observations from Firkins *et al.* (1984).

[1] Basal diet supplemented with urea (U), wet (WDG) or dry (DDG) distillers grains, or wet (WCGF) or dry (DCGF) corn gluten feed.

The prediction of ammonia concentration was compared with observed values in Figure 3C. Generally, the concentration was seen to be overpredicted with an error of 81% of the observed mean, with 27% and 55% of MSPE contributed by overall bias of predictions and deviation of the regression slope from one, respectively. This error was inflated by the high overprediction for the experiment of Robinson & Kennelly (1990). Ammonia concentration was not reported in the experiment of Firkins *et al.* (1984), but predicted ammonia concentration (high for urea treatment, moderate for the corn gluten treatments and somewhat lower for the distillers grain treatments) reflected the properties of the supplements used in terms of nitrogen solubility and degradability (Table 4). In the experiment of Beever *et al.* (1990) ammonia concentration was underpredicted for Diets C and FM1, so that the model predicted an increasing response to an increasing level of fish meal, whereas experimental results showed a response only to the higher level. The concentration of ammonia is the net result of ammonia production from feed, fermentation of protein or hydrolysis of urea, absorption through the rumen wall and passage out of the rumen and utilization by microbes. However, the processes of urea transfer across the rumen wall and in saliva and of ammonia absorption are also influenced by nitrogen metabolism in the tissues of the host animal. Being outside the model, these metabolism processes are only represented indirectly by a function of dietary nitrogen that is assumed to have a correlation with plasma nitrogen concentration (equation 4.12, Mathematical appendix, Dijkstra *et al.* 1992). This indicates the importance of the interrelationships between ruminal processes and tissue metabolism in the determination of nitrogen flows and levels in the rumen.

Evaluation of lipid representation

None of the experiments used to challenge the model included measurements of duodenal lipid flow. In the model, the assumption is made that fatty acids arising from dietary lipids are not incorporated into microbial lipids. However, it has been shown that rumen microorganisms utilize preformed long chain fatty acids to synthesize lipids (Hespell & Bryant 1979). Bauchart *et al.* (1985) indicated that in general, the amount of lipids flowing into the small intestine exceeds the amount ingested with the feed by 10 to 90%. On the data sets used for validation, the excess of lipid outflow ranged from 26 to 88% except for urea treatment (165.7%) in Firkins *et al.* (1984). These values indicate that the level of representation of lipid dynamics in the model is sufficient for the objective. Also, the results suggest that microbial lipid formation from preformed fatty acids is not of major importance in the rumen in most diets. As discussed before, predictions for diets with high lipid-contents diets might not be adequate because they would not reflect the depressing effect of large amounts of fatty acids on fibre digestion and microbial growth.

Volatile fatty acid production and concentration

The prediction of total VFA concentration (excluding valerate and higher acids) and the molar proportions of acetic, propionic, and butyric acids are shown in Figure 4. Overall the prediction of VFA concentration was satisfactory (Figure 4A; error 11% of the observed mean; 82% of MSPE attributed to the random variation about the regression line), but not so for the molar proportions: acetate was overpredicted (Figure 4B; error 9%; overall bias made the highest contribution (68%) to the MSPE), propionate showed high prediction error (Figure 4C; MSPE was 25%, the disturbance proportion (80%) dominating the decomposition), butyrate predictions have a restricted range of values and butyrate was usually underpredicted (Figure 4D; MSPE of 39%; overall bias and deviation of the regression slope from one contributing 53 and 28%, respectively), and valerate was always greatly overestimated (178%). Thus, although qualitative simulated behaviour of VFA molar proportions within experiments is satisfactory, quantitatively predictions need to be improved. The representation of VFA production does not allow for the many possible alternative populations of microbes and biochemical pathways involved in VFA production. The incorrect predictions of molar VFA proportions can be explained by this in all but a few of the diets, because the digestion of feed components is generally well predicted.

In the experiments of Rogers & Davis (1982a and 1982b) in which VFA production was measured by isotope-dilution techniques, predicted molar proportions of individual VFA concentrations were good for Diets HC and LC, but less so for Diet MC. Valerate was severely overestimated by the model (365%). Because the production of 1 mol of valeric acid requires twice as much hexose as the production of 1 mol of acetic or propionic acid, this overestimation will reduce the predicted total amount of VFA produced. If valerate is not taken into account the results are far

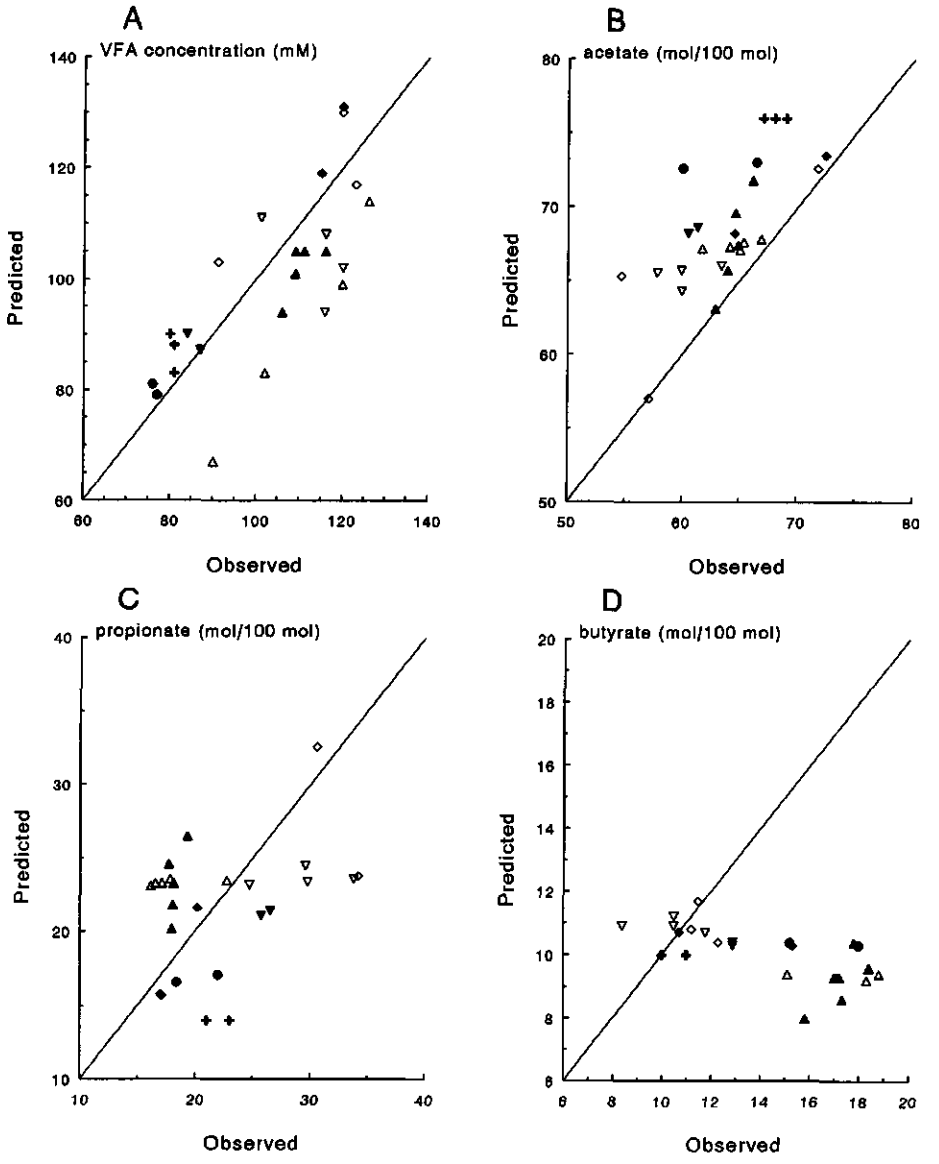

Figure 4. Comparison of observed and predicted values of total volatile fatty acid (VFA) concentration (graph A) and molar proportions of acetate (B), propionate (C) and butyrate (D). See footnote to Figure 2 for key to experiment symbols.

Table 5. *Comparison of experimental observations (obs) with model predictions (pred) of volatile fatty acid (VFA) production, concentration and molar proportion for steers or cows fed mixed diets*

Treatment[1]	VFA concentration		Molar proportions of VFA in rumen						VFA production		Molar proportions of VFA produced					
			Acetate		Propionate		Butyrate				Acetate		Propionate		Butyrate	
	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred
	mM		*mol/100 mol*						*mol/d*		*mol/100 mol*					
Diet MC	120	130	54.7	65.3	34.2	23.8	11.2	10.8	14.3	16.6	51.3	63.1	33.8	25.1	14.9	11.8
Diet HC	123	117	57.1	57.0	30.6	32.6	12.3	10.4	48.3	32.1	56.5	55.1	29.4	33.5	14.1	11.3
Diet LC	91	103	71.7	72.6	17.1	15.7	11.5	11.7	33.3	28.4	62.5	70.7	17.7	16.7	19.8	12.5
								%								
Error %	8.7		10.0		22.6		9.7		30.8		14.7		20.7		29.9	
	mM		*mol/100 mol*						*mol/d*		*mol/100 mol*					
Diet LR	115	119	64.5	68.2	20.2	21.6	15.3	10.3	62.1	49.7	64.2	66.1	20.6	22.8	15.2	11.1
Diet HR	120	131	72.4	73.5	17.0	15.8	10.7	10.7	71.4	58.7	70.8	71.1	18.6	17.1	10.5	11.8
								%								
Error %	7.0		4.0		7.0		27.2		18.8		2.0		9.6		23.7	

Observations from Rogers & Davis (1982a) (Diet MC), Rogers & Davis (1982b) (Diets HC and LC) and Lebzien (1980) (Diets LR and HR).

[1] Diet MC, 50% concentrate, 50% roughage; Diet HC, 75% concentrate, 25% roughage; Diet LC, 18% concentrate, 82% roughage; Diet LR, 55% concentrate, 45% roughage; Diet HR, 13% concentrate, 87% roughage.

very well for propionate but not so well for acetate and butyrate (Table 5). The differences increased with increasing roughage content in these diets, which is not in agreement with the claims of Sutton (1985) for diets fed to sheep. Prediction of total VFA production was overestimated by 16% for Diet MC and underestimated by 34% and 15% for Diets HC and LC, respectively, which is in agreement with Sutton (1985), who stated in his review that VFA production results for concentrate diets are more variable than results for roughage diets.

In the experiment by Lebzien (1980), valerate molar proportion of VFA concentration was overestimated with an error 2.31 times the observed mean. If valerate was not taken into account, only butyrate on diet LR was underestimated (Table 5). For observed results, the molar proportions of VFA produced were strongly related with molar proportions in concentration on both diets, partially because butyrate production was calculated, rather than measured, from acetate and propionate measured production corrected for interconversion of acetate and propionate to other VFA, by an average amount and molar percentage in concentrations. The model underpredicted total VFA production by 20 and 18%. In evaluating the agreement between model predictions and observed values, it should be noted that in a review of VFA production, measured by isotope-dilution techniques, and using a range of diets, production of acetic acid (1.5-8.3 mol/kg digestible DM) and propionic acid (0.8-3.8 mol/kg digestible DM) varied widely, and Sutton (1985) indicated that some of this variation was due to measurement errors. Equally, Lebzien (1980) found that the method of sampling (by hand or automatic sampling ventral rumen sac) had a pronounced effect on VFA production rates, which were on average 80% higher when the automatic sampling method was used. Observed values for VFA production rates in Table 5 were obtained by the automatic sampling method, and this could add an explanation of model underprediction. The results given in Table 5 are well within the experimental range reported by Sutton (1985); yet VFA molar proportions for other diets used to validate the model (Figure 4) are generally not predicted satisfactorily.

In analyzing the problems encountered with prediction of VFA molar proportions, it should be noted that simulation of ruminal digestion of ingested substrates and microbial metabolism was in general satisfactory at the level of aggregation adopted. Thus, it is not likely that discrepancies in predicted VFA molar proportions can be explained by discrepancies in predicted ruminal digestion of substrates. Another possible error arises from assumed stoichiometric coefficients for lactate fermentation. However, lactate is absent in most of the diets used to challenge the model or present in small quantities only. These considerations indicate that the stoichiometric coefficients derived by Murphy *et al.* (1982) by statistical analysis of a large data set are not applicable to the diets used for evaluation in combination with the concepts of the model. Several reasons might be put forward to explain this. First, the comparison experiments are mainly on dairy cattle, whereas the data set used by Murphy *et al.* (1982) almost completely concerned experiments with sheep or beef cattle. Second, the considerable amount of VFA produced from engulfment of microbes or derived from recycled nutrients within the rumen was not taken into

account. Third, both pH and type of VFA can significantly affect absorption rates of VFA from the rumen (Thorlacius & Lodge, 1973), which might invalidate the assumption in Murphy *et al.* (1982) that rumen VFA concentrations reflect their relative production rates. Finally, although Murphy *et al.* (1982) accounted for differences in rumen fluid characteristics by developing coefficients for roughage and for concentrate diets, the shift in fermentation pathways at low pH values within concentrate diets (Strobel & Russell, 1986) was not accounted for. Argyle & Baldwin (1988) did adjust stoichiometric coefficients for fermentation of sugars and starch at pH values lower than 6.2 and showed effects on VFA molar proportions. Unfortunately, these authors reported that insufficient data prevented challenge of the adjustments. For the diets used to validate the model and with an average pH below 6.2, the suggested adjustments would yield variable results. With the high intake level diet of Robinson *et al.* (1986), application of these adjustments might decrease acetate overprediction and butyrate underprediction, but would inflate propionate overprediction. In the experiment of McCarthy *et al.* (1989) experiment, prediction of molar proportions of acetate and propionate might improve, but that of butyrate would deteriorate. In the experiment of Robinson & Kennelly (1990) experiment, predictions of molar proportions of all VFA would improve. Finally, butyrate underprediction would be decreased and acetate and propionate prediction error would be inflated for the high concentrate diet of Rogers & Davis (1982b). Thus, the prediction of molar proportions of VFA merits further study.

In conclusion, the objective of the model to examine the profile of nutrients available for absorption from diets containing forage has mainly been achieved. The prediction of NDF and total NAN outflow and VFA concentrations are satisfactory at the level of aggregation of the model, for a wide range of diets. The incorporation of the composition of microbes with varying polysaccharide content has responded well to the different feeding regimens. Detailed evaluation of the model and indeed confidence in some of the model parameters are limited by the problems of measuring duodenal flows and VFA production. This problem is particularly evident with the predictions of microbial protein flow and VFA molar proportions and is not surprising in view of the large number of microbial species present in the rumen. This finding is in agreement with earlier rumen models and again demonstrates the need for more quantitative work on this aspect of ruminant nutrition before improvements in the prediction of nutrient supply can be achieved.

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Chapter 3

ABSORPTION OF VOLATILE FATTY ACIDS FROM THE RUMEN OF LACTATING DAIRY COWS AS INFLUENCED BY VOLATILE FATTY ACID CONCENTRATION, pH AND RUMEN LIQUID VOLUME

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The effect of rumen liquid volume, pH and concentration of volatile fatty acids (VFA) on the rates of absorption of acetic, propionic and butyric acids from the rumen was examined in lactating dairy cows. Experimental solutions introduced into the emptied, washed rumen comprised two different volumes (10 or 30 l), four levels of pH (4.5, 5.4, 6.3, 7.2) and three levels of individual VFA concentrations (20, 50 or 100 mM-acetic, propionic or butyric acid). All solutions contained a total of 170 mM-VFA and an osmotic value of 400 mOsmol/l. Absorption rates were calculated from the disappearance of VFA from the rumen corrected for passage with liquid phase to the omasum. An increase in initial fluid pH caused a reduction in fractional absorption rates of propionic and butyric acids. Increasing the initial pH from 4.5 to 7.2 reduced fractional absorption rates of acetic, propionic and butyric acid from 0.35, 0.67 and 0.85 to 0.21, 0.35 and 0.28 /h respectively. The fractional absorption rates of all VFA were reduced ($P < 0.05$) by an increase in initial rumen volume. The fractional absorption rate of acetic acid was lower ($P < 0.05$) at an initial concentration of 20 mM than of 50 mM. The fractional absorption rate of propionic acid tended ($P < 0.10$) to decrease as the level of concentration increased while fractional absorption rate of butyric acid was not affected by butyric acid concentration. These results indicate that relative concentrations of VFA in rumen fluid might not represent relative production rates and that attempts to estimate individual VFA production from substrate digestion must take account of pH and VFA concentration.

Volatile fatty acids: Dairy cows

In the rumen of dairy cows, volatile fatty acids (VFA) are produced by microbial fermentation of carbohydrates and protein. Acetic, propionic and butyric acids are the predominant VFA occurring in the rumen fluid, their concentration and relative proportions related to the level of feed intake (Sutton, 1985) and the composition of the diet (Murphy *et al.* 1982). In ruminants, VFA constitute the major source of energy, providing at least 50% of total amount of digested energy (Sutton, 1985). Both the yield of total VFA and the type of VFA formed can significantly affect the utilization of absorbed nutrients in dairy cows and, thus, can affect milk volume and composition to a considerable extent (Thomas & Martin, 1988).

Various methods have been applied to estimate or predict the amount of individual

VFA available from rumen fermentation. Production rates of VFA as measured by isotope-dilution techniques showed a wide variability and errors of measurement have been discussed (Sutton, 1985). Due to metabolism of VFA by the rumen mucosa, estimates of VFA availability by measurement of the amounts of VFA appearing in the portal system are considerably lower than those measured by the previous method, and the applicability of the results has been questioned (Bergman, 1990). A third approach is to estimate the amount of available VFA by prediction from the amount of substrate fermented in the rumen, multiplied by stoichiometric fermentation variables for these substrates (Koong *et al.* 1975; Black *et al.* 1981; Murphy *et al.* 1982). The accuracy of this approach depends, amongst other aspects, on the relationship between VFA production and VFA concentration in the rumen fluid, so quantitative information on the rate of absorption of individual VFA from the rumen is required. Several experiments have been performed to study this rate of VFA absorption. The effects of chain length, pH, effective absorptive surface area, osmolality and concentration on individual VFA absorption rates have been reported (for reviews, see Bugaut, 1987; Bergman, 1990). However, results are conflicting, probably because of differences in experimental methods applied (Carter & Grovum, 1990). In particular, *in vitro* studies give qualitative rather than quantitative results and results are often not applicable to the normal functioning animal (Bergman, 1990). Also, the vast majority of these experiments have been performed with sheep and between-species differences in rates of absorption might exist (Bugaut, 1987).

The objective of this study was to quantify the effects of rumen liquid volume, pH and concentration of VFA on the fractional rates of absorption of acetic, propionic and butyric acids from the rumen of lactating dairy cows.

MATERIAL AND METHODS

Animals and diets

Two 7-year old lactating Black and White cows (crossbred Friesian/Holstein-Friesian) fitted with large ruminal cannulas (Bar Diamond, Idaho, USA) were used. The cows were 2-3 months in lactation at the start of the experiment. The average milk production of the cows during the experimental period (2 months) was 16.5 kg/d. Cows grazed together with the other cows in the herd, and in addition received 2 kg of commercial concentrate during the experimental period.

Experimental design and procedures

A total of twelve experimental solutions were prepared containing three levels of individual VFA concentration (20, 50 or 100 mM-acetic, propionic or butyric acid) and four levels of pH (4.5, 5.4, 6.3 or 7.2) which were introduced to the rumen at two different volumes (10 or 30 l). The solutions were based on McDougalls buffer (McDougall, 1948) and the composition of the solutions is given in Table 1. All solutions contained 170 mM-VFA. The pH of the solutions was set at the desired

level by addition of NaOH or HCl and if necessary NaCl was added to give an osmolality of approximately 400 mOsmol/l. Fractional outflow rate of rumen fluid was estimated utilizing cobalt ethylenediaminetetraacetic acid (CoEDTA) as a marker (70 mg CoEDTA (140 g/kg)/l; Uden *et al.* 1980) .

During the experimental period (2 months), the cows were confined in a stall after the morning milking every Monday, Wednesday and Friday. Rumen ingesta were removed and stored in an insulated container before return. Once the rumen had been washed four times with warm water, it was washed twice more with five litres of the experimental solution at body temperature. Next, either 10 or 30 litres of the solution were introduced to the rumen and left for 60 min. Samples were taken immediately and every 10 min after introduction of the solution for determination of pH, osmolality and concentration of Co and VFA of rumen fluid. After 60 min, the fluid was removed and the withdrawn volume measured. Each combination of pH (n = 4), VFA concentration (n = 3) and volume (n = 2) of the solution was assigned randomly to one of the days. However, in the pre-experimental period it was shown that the pH of the solutions increased rapidly after introduction to the rumen. Hence in the experimental design, six of the twelve solutions with a pH of 6.3 or 7.2 were replaced by solutions with a pH of 4.5 or 5.4.

In all samples pH was measured immediately after collection. Fluid osmolality was determined within three hours after collection by freezing-point depression (Knauer half micro-osmometer) in a portion of the samples taken at 0 and 60 min after introduction of the solution. Co concentration was determined in another portion of these samples using an atomic absorption spectrophotometer (Varian Spectra-300). A portion (10 ml) of the samples was acidified with 0.5 ml phosphoric acid (850 ml/l)

Table 1. *Composition of the experimental solutions*

	Concentration (mM) in solution with initial pH of:			
	4.5	5.4	6.3	7.2
Na_2HPO_4	39	39	39	39
$NaHCO_3$	70	70	70	70
NaCl	63	39	14	5
KCl	4.6	4.6	4.6	4.6
$CaCl_2$	0.9	0.9	0.9	0.9
$MgCl_2$	0.7	0.7	0.7	0.7
HCl	14	-	-	-
NaOH	-	6	10	14
VFA	170	170	170	170

Concentration of acetic, propionic and butyric acids in solution 1, 2 and 3 respectively: 100, 50, 20 mM; 20, 100, 50 mM; 50, 20, 100 mM; VFA, volatile fatty acid.

and frozen at -15° until analyzed for VFA concentrations by gas-liquid chromatography (Packard Becker, model 419, glass column filled with chromosorb 101, carrier gas (N_2) saturated with formic acid at approximately 190°C, isovaleric acid was used as the internal standard).

Calculation of results and statistical analysis

The water inflow, fractional outflow rate of fluid, and fractional absorption rates of acetic, propionic and butyric acids, were calculated using the differential equations:

$$dCo/dt = -k_l Co \quad (1)$$

$$dV/dt = -k_l V + I \quad (2)$$

$$dVFA/dt = -(k_l + k_r)VFA \quad (3)$$

where Co is the amount of cobalt in the rumen (g); V is rumen fluid volume (l); I is constant inflow of water to the rumen (l/h); VFA is amount of acetic, propionic or butyric acid in the rumen (mmol); k_l is fractional liquid passage rate (/h); k_r is fractional absorption rate of VFA (/h); t is time (h). The analytical solutions to equations 1-3 are as follows:

$$Co = Co(0)\exp(-k_l t) \quad (4)$$

$$V = (V(0) - I/k_l)\exp(-k_l t) + I/k_l \quad (5)$$

$$VFA = VFA(0)\exp[-(k_l + k_r)t] \quad (6)$$

where Co(0), $V(0)$ and VFA(0) denote the amount of Co, and the volume and the amount of VFA, respectively, immediately after introduction of the solution to the rumen. Since the initial and the end values of Co, V and VFA have been determined, the inflow of water, fractional passage and absorption rates can be calculated.

Data were subjected to least squares analysis of variance by the general linear model procedure (SAS Institute Inc., 1985), with results expressed as least square means, using the general model:

$$y_{ijkl} = \mu + A_i + P_j + V_k + S_l + e_{ijkl} \quad (7)$$

where μ is the overall mean, A_i, P_j, V_k and S_l are the animal, pH, volume and solution composition effect respectively, and e_{ijkl} is the residual term. Effect of day of introduction of experimental solutions was detected ($P < 0.05$) only for the initial and end osmolality of rumen fluid using the procedures as described by Gill & Hafs (1971), and after examination of uniformity of correlations between data for any two d, was dropped for all the other variables. Of all possible interaction terms amongst treatments, a volume x pH interaction was detected ($P < 0.05$) only for the end pH

of rumen fluid and, hence, for all other variables, interactions were not included in the analysis. Means were separated using the Tukey test.

Finally, a non-linear equation was fitted by the non-linear regression procedure (SAS, 1985) to the data corrected for dilution and outflow of VFA with the fluid, to estimate the amount of each VFA absorbed in relation to the pH, concentration and volume:

$$T = v_m V^{0.75}/[(1 + (K/C_{VFA})^{\Phi})(1 + (pH/J)^{\Theta})] \quad (8)$$

where T is the amount of each VFA absorbed per unit of time (mmol/h); v_m is maximum absorption rate (mmol/(l·h)); $V^{0.75}$ is metabolic volume of the rumen, representing the absorptive surface (l); K is affinity constant for absorption of VFA (mM); C_{VFA} is concentration of VFA in rumen fluid (mM); J is inhibition constant for absorption of VFA related to pH of rumen fluid; Θ, Φ are sigmoidal steepness variables. This equation was formulated based on two assumptions. First, at high rumen concentrations, absorption can be limited as the VFA accumulate within the receiving cell or if the availability of ions for co- or countertransport is limiting, while low concentrations will limit absorption as the concentration gradient decreases. Both can be represented simultaneously sigmoidally in the first part of the denominator (Thornley & Johnson, 1990). Second, since the absorption of dissociated VFA is negligible, pH will affect absorption rates by affecting the dissociated:undissociated VFA ratio according to the Henderson-Hasselbalch equation. This is represented in the second part of the denominator.

RESULTS

Values in Table 2 refer to the rumen fluid indices as affected by initial pH, composition and volume of the experimental solution. The fractional fluid passage rate was not affected by pH nor volume, but the composition of the solution had a tendency ($P < 0.10$) to affect the outflow rate. For all treatments there was a net inflow of water to the rumen. With increasing levels of initial pH the net fluid inflow tended to increase. Similarly, the net fluid inflow was significantly higher ($P < 0.05$) with the higher initial volume. The fluid end volume is a result of the initial volume, passage rate and inflow of water. Thus, both initial volume ($P < 0.01$) and pH ($P < 0.05$) significantly affected the fluid end volume. After one hour, rumen fluid pH reached an end value of 8.0 - 8.2, except for the solutions with an initial value of pH 4.5. The end pH was significantly lower ($P < 0.05$) for the higher initial liquid volume. Average initial fluid osmolality was 392 mOsmol/l and was not related ($P > 0.10$) to either initial pH, volume or composition of the solution. After 60 min, fluid osmolality was decreased to an average 288 mOsmol/l. Low levels of both initial pH and volume resulted in low osmolality values. Again, there was no effect ($P > 0.10$) of composition of the solution.

Table 2. *The effect of initial pH, composition and volume of experimental solution on rumen fluid indices in lactating dairy cows*

	pH				Solution[1]			Volume (l)		Statistical significance of:			
	4.5	5.4	6.3	7.2	1	2	3	10	30	pH	S	V	RSD[2]
Fractional fluid passage rate (/h)	0.21	0.26	0.26	0.25	0.31	0.22	0.21	0.28	0.22	NS	*	NS	0.13
Net fluid inflow (l/h)	9.1	11.8	13.1	13.4	13.1	11.7	10.7	10.4[a]	13.3[b]	**	NS	**	3.7
Fluid end volume (l)	24.5	26.4	27.0	27.4	26.1	26.7	26.3	16.6[a]	36.1[b]	**	NS	***	2.3
Fluid end pH	7.13[a]	8.01[b]	8.22[b]	8.23[b]	7.57	7.81	7.53	8.20[a]	7.38[b]	***	NS	***	0.19
Fluid end osmolality (mOsmol/l)	278	293	291	298	293	283	294	283[a]	297[b]	*	NS	**	16.6

pH, effect of initial pH of solution; S, effect of volatile fatty acid composition of solution; V, effect of volume of solution; RSD, residual standard deviation; NS, not significant ($P > 0.10$).

[a,b] Means within rows and treatments with different superscript letters were significantly different ($P < 0.05$).

* $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$.

[1] Concentrations of acetic, propionic and butyric acid in solution 1, 2 and 3 respectively: 100, 50, 20 mM; 20, 100, 50 mM; 50, 20, 100 mM.

[2] 40 df, except fluid end pH (37 df) and fluid end osmolality (34 df).

Fractional absorption rates of acetic, propionic and butyric acids, as influenced by initial pH, composition and volume of the experimental solution, are given in Table 3. Fractional absorption rates of propionic and butyric acid were significantly ($P < 0.05$) higher with low initial pH levels, but the fractional absorption rate of acetic acid was not significantly ($P > 0.10$) affected by initial pH, although the estimated absorption rate tended to decrease with an increase in pH. Fractional absorption rates of acetic, propionic and butyric acids were compared (Tukey test) within initial pH levels using the pooled average standard deviation (Snedecor & Cochran, 1968). Fractional absorption rates of the VFA with an initial pH of 4.5 were, in order ($P < 0.05$): butyric acid > propionic acid > acetic acid; with an initial pH of 5.4, this order was: butyric acid = propionic acid > acetic acid; and with initial pH values of 6.3 and 7.2 no significant ($P > 0.05$) differences between absorption rates of the VFA were detected. The composition of the solution had a significant ($P < 0.01$) effect on the fractional absorption rate of acetic acid, the intermediate level of concentration (50 mM) being significantly higher than the low level of concentration (20 mM). Fractional absorption rate of propionic acid tended ($P < 0.10$) to increase with decreasing concentrations of propionic acid in the solution, but fractional absorption rate of

butyric acid was not significantly ($P > 0.10$) affected by the concentration of the acid. For all VFA, fractional absorption rates were significantly ($P < 0.05$) higher with the 10 litre solution than the 30 litre solution.

The effects of concentration and pH on the amount of each VFA absorbed are illustrated in Figs. 1 and 2. In Fig. 1 the amount of VFA absorbed as estimated by equation 8 at pH 6.0 is presented: in Fig. 2 the estimated amount of VFA absorbed at concentration 50 mM is presented. The estimates of the variables suggest a higher potential maximum absorption rate for butyric acid than the other acids, and a higher potential maximum absorption rate for propionic acid than acetic acid. The individual pH values for half-maximum speed (J in equation 8) were very close to each other and a constraint on the estimation of this variable was added to ensure the same value for each VFA. The estimate of this variable was 6.0. The sigmoidal steepness variable for the concentration effect (Φ) significantly reduced the residual variation for acetic acid only, indicating a weak sigmoid behaviour for this acid. The absorption of VFA showed a more pronounced sigmoidal behaviour in response to changes of the rumen fluid pH, the steepness variable (Θ) being highest for butyric and lowest for acetic acid. Hence, with a decrease in pH, absorption of butyric acid will increase more than the other acids and absorption of propionic acid more than acetic acid.

Table 3. *The effect of initial pH, composition and volume of experimental solution on fractional absorption rates of volatile fatty acids in lactating dairy cows*

	pH				Concentration (mM)			Volume (l)		Statistical significance of:			
	4.5	5.4	6.3	7.2	100	200	300	10	30	pH	S	V	RSD[1]
Fractional absorption rate of (/h):													
Acetic acid	0.35	0.35	0.33	0.21	0.32^{ab}	0.43^{a}	0.18^{b}	0.37^{a}	0.25^{b}	NS	***	**	0.20
Propionic acid	0.67^{a}	0.54^{ab}	0.51^{ab}	0.35^{bc}	0.44	0.51	0.60	0.62^{a}	0.41^{b}	**	*	***	0.20
Butyric acid	0.85^{a}	0.53^{b}	0.46^{b}	0.28^{b}	0.54	0.45	0.60	0.60^{a}	0.45^{b}	***	NS	**	0.24

pH, effect of initial pH of solution; S, effect of volatile fatty acid composition of solution; V, effect of volume of solution; RSD, residual standard deviation; NS, not significant ($P > 0.10$).

[a,b] Means within rows and treatments with different superscript letters were significantly different ($P < 0.05$).

* $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$.

[1] 40 df.

DISCUSSION

In previous studies VFA absorption rates have been examined *in vitro* or using animals in which the reticulo-rumen has been isolated by ligatures, using rumen pouches or resorption chambers. Though allowing precise control and accurate measurements to elucidate mechanisms of absorption, results of these studies are not directly

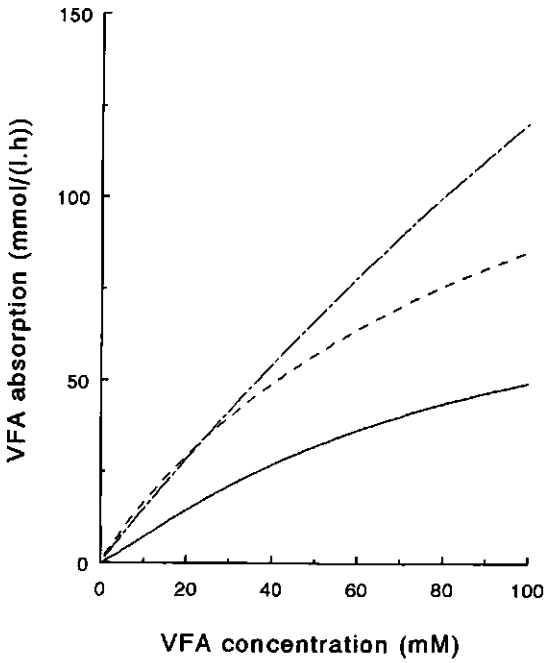

Figure 1. Influence of concentration of acetic, propionic or butyric acid on the absorption of acetic (————), propionic (----------) and butyric (— · — · —) acids at pH 6.0 for lactating dairy cows calculated from equation 8:

$$T = v_m V^{0.75}/[(1 + (K/C_{VFA})^{\Phi})(1 + (pH/J)^{\Theta})]$$

where T is the amount of each VFA absorbed per unit of time (mmol/h); v_m is maximum absorption rate (mmol/(l·h)); $V^{0.75}$ is metabolic volume of the rumen, representing the absorptive surface (l); K is affinity constant for absorption of VFA (mM); C_{VFA} is concentration of VFA in rumen fluid (mM); J is inhibition constant for absorption of VFA related to pH of rumen fluid; Θ, Φ are sigmoidal steepness variables.

VFA	v_m	K	J	Φ	Θ	R^2	RSD
Acetic acid	172.0	79.1	6.02	1.17	3.91	0.74	18.9
Propionic acid	356.2	112.0	6.02	0.95	4.61	0.82	20.3
Butyric acid	1391.2	493.4	6.02	0.99	5.13	0.89	18.2

where R^2 is coefficient of determination and RSD is residual standard deviation. For details of equation 8, see p. 75.

applicable to the normal lactating dairy cow (Stevens, 1970; Bergman, 1990). The present study used intact, lactating dairy cows to allow estimations of VFA absorption rates which would be generally applicable to dairy cattle.

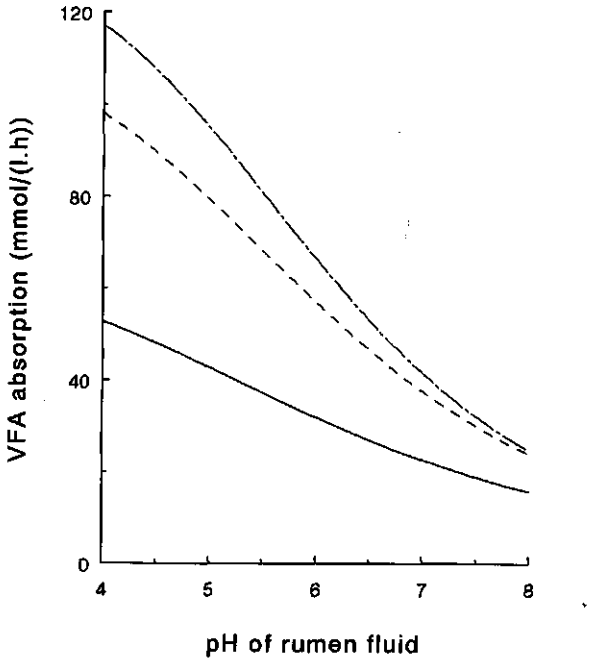

Figure 2. Influence of pH of rumen fluid on absorption of acetic (————), propionic (----------) and butyric (— · — · —) acids at a concentration of each acid of 50 mM for lactating dairy cows calculated from equation 8 of which variables are given in Figure 1.

Fluid dynamics and osmolality of fluid

Clearance of VFA from the rumen is either by passage with the fluid to the omasum, or by absorption through the rumen wall. For this reason it is important to quantify the rumen fluid dynamics related to the pH, composition and volume of solutions introduced to the rumen. In a recent review Carter & Grovum (1990) suggested that the mechanisms governing the movements of water to and from the rumen operate to minimize insults to the osmotic balance between rumen and body fluids, with a significant amount of water inflow determined at osmolality values higher than 370 mOsmol/l. Indeed, in the present study the net water inflow appeared to be related to the osmotic pressure of the rumen fluid, giving rise to differences in end volume of the rumen fluid since the fractional liquid passage rate was not affected by pH, composition or volume of the experimental solution. The VFA are major determinants of rumen fluid osmotic pressure, both directly and by co- or countertransport of other ions. Absorption of VFA has been shown to be linked to HCO_3^- secretion into the rumen fluid in the ratio 2:1 (Masson & Phillipson, 1951; Ash & Dobson, 1963; Gäbel

et al. 1989). Hence, increments in fractional absorption rates of VFA due to decreases in pH or in rumen volume (Table 3) will result in a decrease of the rumen fluid osmotic pressure (Table 2). Both the absorption of VFA into rumen epithelial cells and the concomitant secretion of HCO_3^- will contribute to the increase in pH and explain the observed lower end pH at the higher volume level. From a summary of several studies Warner & Stacy (1972) showed that a zero net flux of water across the rumen wall occurred at osmolality values in the range of 295-360 mOsmol/l. Considering the relation between osmotic pressure and fluid dynamics it might be suggested that the relative net water flux decreased with time after introduction of the solution, since the osmotic pressure decreased from an initial value of approximately 400 mOsmol/l to an end value of 290 mOsmol/l. Before one h was over water inflow may have become negative. However, in the calculations a constant inflow of water during the hour was assumed. Hence, VFA absorption rates may have been overestimated in the first period after introduction and underestimated in the later period. However, saliva also contributes to water inflow and it was observed that animals were salivating from the moment of introduction until removal of the solution. Equally, high osmotic values of rumen fluid have been shown to decrease VFA fractional absorption rates (Oshio & Tahata, 1984) and, conceivably, this effect of osmotic pressure might balance to some extent the effect of assuming a constant water inflow on absorption rates.

VFA absorption

In a number of studies the effects of pH and osmolality of fluid, effective surface area, type and concentration of VFA on absorption of VFA have been reported (for reviews, see Bugaut, 1987; Bergman, 1990). Based on these results several models of VFA absorption have been proposed (Ash & Dobson, 1963; Stevens, 1970; Argenzio, 1988; Gäbel, 1990). In these models VFA absorption is explained to a large extent by diffusion, the rate affected by pH of the fluid at the lumen side, cell contents and intracellular metabolism of VFA. Except for the Stevens (1970) model, all models assume transport systems across the rumen epithelium supplying protons for the conversion of a VFA anion to the acid form. The present study gives considerable support to the model of VFA-coupled exchange of protons. Ionized components do not diffuse passively across the rumen cell membrane (Bugaut, 1987; Gäbel, 1990). Yet even at pH levels above neutral (pH 7.0) and, hence, according to the Henderson-Hasselbalch equation, more than 99% of each VFA present is in the dissociated form, substantial VFA fractional absorption rates were calculated (Table 3). Moreover, the estimated pH value for half-maximum rate of VFA absorption was 6.0 (Fig. 1) and at this pH value only 6% of each VFA is present in the dissociated form. It is obvious that protons have been supplied to the dissociated VFA to establish such relatively high fractional absorption rates, probably in an acid microclimate near the rumen wall (Bugaut, 1987). Both the present study and the work reported by Danielli *et al.* (1945), Tsuda (1956), Aafjes (1967), Weigand *et al.*

(1972), Thorlacius & Lodge (1973) and MacLeod & Ørskov (1984) found decreasing pH levels to increase fractional absorption rates. Furthermore, the present study showed that increased fractional absorption rates were significantly affected by the type of VFA absorbed (Table 3). Similar pH x type of VFA interactions on clearance of VFA have been reported by a number of authors (Danielli *et al.* 1945; Pfander & Phillipson, 1953; Tsuda, 1956; Hogan, 1961; Aafjes, 1967; Weigand *et al.* 1972; Thorlacius & Lodge, 1973; Oshio & Tahata, 1984), indicating that at alkaline pH levels relative rates of absorption of the three VFA do not differ to such a great extent as at acid pH levels.

Several authors reported the absorption of the major VFA as a direct relationship to their rumen concentration (Masson & Phillipson, 1951; Tsuda, 1956; Hogan, 1961; Thorlacius & Lodge, 1973; Weigand *et al.* 1972; Oshio & Tahata, 1984), although the absorption of valeric acid decreased as rumen fluid concentration of valeric acid increased (Oshio & Tahata, 1984). In contrast, in the present study the fractional absorption rate of acetic acid was decreased at the lowest concentration level (20 mM) and also tended to decrease at the highest concentration level (100 mM). The fractional absorption rate of propionic acid tended to decrease with an increase in the concentration. In attempting to reconcile this result one must remember first, that absorption of VFA is a diffusion process and the rate is dependent upon, amongst others, the concentration gradient, and second, that propionic and butyric acid are extensively metabolized by the rumen epithelial tissue. The latter mechanism is reflected in the relatively low levels of propionic and butyric acid in the portal blood of ruminants (Bergman, 1990). Thus, the low rumen acetic acid concentration achieved with an initial concentration of 20 mM (the minimum values observed were 6 mM) and the relatively high concentration of acetic acid in the blood will result in a rather low concentration gradient and, hence, decrease the fractional absorption rate. As the capacity of the epithelial cell for VFA can be saturated (Bergman, 1990), at high levels of propionic acid in the rumen fluid the metabolism of this acid in the cell might contribute less to the clearance of propionic acid from the rumen, decreasing the fractional absorption rate. However, the reason for butyric acid fractional absorption rate not being affected by the concentration is not apparent. It should be noted also that both the lowest acetic acid concentration and the highest propionic and butyric acid concentrations are outside the normal physiological range measured in rumen fluid of dairy cattle.

As well as the previously mentioned factors, the quantity of VFA absorbed from the rumen depends on the effective surface area (Dobson, 1984; Bugaut, 1987). However, none of the reports on VFA absorption from the rumen studied the effect of the liquid volume on clearance rates. In the present study absorption rates were significantly affected by the volume of the experimental solution introduced. Since an increase in liquid volume is expected to increase the average distance of a solute from the rumen mucosa and hence decrease the diffusion rate, the influence of the absorptive epithelium can be inferred from these findings. The present results indicate

that the effect of volume and surface area could be represented by metabolic volume (volume$^{0.75}$). This was confirmed by analysis of the experimental data using equation 8, in which the 0.75 term was replaced by an independent variable. The estimation of this independent variable was 0.69. The absorptive surface in relation to the liquid volume will have an effect on the pathway (absorption across the rumen wall or passage to the omasum) of VFA disappearance from the rumen as well. In sheep (Sutherland, 1963; Von Engelhardt & Hauffe, 1975) and calves (Edrise & Smith, 1977), the estimated contribution of passage was 10-20% of the total disappearance from the rumen. In cattle this figure was estimated to be 30% at maintenance level and 40% at four times maintenance (Tamminga & Van Vuuren, 1988). Although differences in pH and fractional passage rates could explain some of these species and intake level differences on the contribution of passage to VFA clearance, it is clear that the generally higher liquid volume in cattle contributes substantially to an explanation of these differences, since unlike absorption, passage of VFA to the omasum is assumed to increase linearly with an increase in the total amount of VFA present in the rumen. In turn, at a higher rumen liquid volume, this relatively reduced clearance rate of VFA per unit volume might reduce rumen capacity and *ad lib.* feed intake (Tamminga & Van Vuuren, 1988). By extrapolation from the volumes used in the present experiment (10 or 30 l), using equation 8, to the volumes in the previously described report (55-80 l, Tamminga & Van Vuuren, 1988), the estimated contribution of passage to VFA disappearance was in the range of 20-35%, slightly less than estimated by Tamminga & Van Vuuren (1988).

The quantitative results of the present study are not readily comparable to other studies of VFA absorption. First, results reported in the literature show a wide range of variation due to differences in the experimental methods applied (Carter & Grovum, 1990; Bergman, 1990). Second, the present study found significant effects of pH, concentration, volume and type of VFA on rate of absorption from the rumen. Previous studies, however, have seldom reported the volume of the fluid in the experiment, or have reported absorption rates using individual VFA concentrations which are often within the range usually found in rumen fluid, or have used solutions with interrelated pH and VFA concentration levels. Clearly, the present study indicates that mathematical models of rumen fermentation which are developed to predict nutrient supply to the animal should consider the volume, concentration, pH and type of VFA in order to predict accurately the dynamics of VFA absorption. Equally, results of the present study suggest that the often reported relative concentrations of VFA in the rumen fluid might not represent the relative production rates, particularly at acid pH levels. From a summary of published values Sutton (1985) concluded that the agreement between molar proportions of VFA produced, as measured by isotope dilution methods, and molar proportions found in the rumen fluid was less variable for high-roughage diets than high-concentrate diets and less variable for sheep than cattle. The different effects of pH on the absorption of VFA in the present study add to an explanation of this variation, as high concentrate diets

support relatively low pH values of rumen fluid and, generally, the pH of rumen fluid in cattle is lower than in sheep. In this regard, unlike recent studies (Koong *et al.* 1975; Black *et al.* 1981; Murphy *et al.* 1982), pH of the rumen fluid and the concentration of each VFA have to be included if predictions of VFA production from substrate digestion in the rumen and VFA molar proportions are to be accurate.

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Chapter 4

SIMULATION OF THE DYNAMICS OF PROTOZOA IN THE RUMEN

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SIMULATION OF THE DYNAMICS OF PROTOZOA IN THE RUMEN

JAN DIJKSTRA

A mathematical model is described that simulates the dynamics of rumen micro-organisms, with specific emphasis on the rumen protozoa. The model is driven by continuous inputs of nutrients and consists of 19 state variables, which represent the nitrogen, carbohydrate, fatty acid and microbial pools in the rumen. Several protozoal characteristics were represented in the model, including preference for utilization of starch and sugars compared with fibre and of insoluble compared with soluble protein; engulfment and storage of starch; no utilization of ammonia to synthesize amino acids; engulfment and digestion of bacteria and protozoa; selective retention within the rumen; death and lysis related to nutrient availability. Parameters were assigned values based on results of appropriate experiments, where available, or alternatively were assigned values on a priority basis, given relevant experimental observations. Sensitivity analyses on diets with a high roughage (R) or a high concentrate (C) proportion indicated that the model is sensitive to bacterial engulfment rate, maintenance requirements of protozoa and death rate of protozoa. Comparisons between model predictions and experimental observations showed reasonable agreement for protozoal biomass in the rumen, but protozoal turnover time was not predicted well. Simulations in which dietary components were independently varied, indicated a rapidly increased protozoal biomass in response to increases in dietary starch content, but further increases in starch content of Diet C caused protozoal mass to decline. Increasing the sugar content decreased protozoa on Diet C, while moderate elevations of the sugar content on Diet R increased protozoal biomass. Simulated protozoal biomass did not change in response to variations in dietary NDF content. Reductions in dietary nitrogen decreased bacterial growth efficiency and total bacterial biomass, and this resulted in an increased protozoal biomass. Depending on the basal intake level and dietary composition, protozoal concentration in the rumen was either increased or decreased by changes in feed intake level. Such changes in relative amounts of protozoal and bacterial biomass markedly affected the supply of nutrients available for absorption. The integration of protozoal, bacterial and dietary characteristics through mathematical representation provided a quantitative understanding of mechanisms of protozoal responses to changes in dietary inputs.

Rumen: Computer simulation: Mathematical model: Protozoa: Ruminants

In ruminants, the quantity and quality of nutrients absorbed from the digestive tract are generally quite different from that ingested because of the activity of the rumen microbial population. The significance of the rumen microorganisms in the rumen digestion processes and consequently in animal production has been elaborated by

many research workers (Hungate, 1966). The role of rumen protozoa in these processes has been subject of much debate and our knowledge of rumen protozoa and their function is far less than knowledge of rumen bacteria. The contribution of protozoa to total rumen microbial biomass may equal that of bacteria (Hungate, 1966), suggesting a possibly important role of protozoa in ruminal fermentation processes. Yet, in numerous experiments, their presence has been demonstrated to be non-essential for the ruminant (e.g. Veira, 1986). Studies on the effects of elimination of protozoa from the rumen have been performed to increase knowledge of the overall effect of protozoa in rumen fermentation, but the modifications brought about as a result of defaunation are not systematic. Since bacterial and protozoal metabolism differs in many aspects, establishment of the role of protozoa in rumen digestion processes and of the effects of defaunation on these processes should include the complex relationships between protozoa, bacteria, animal and dietary factors (Jouany *et al.* 1988). At present, such an integrative approach, with the aim to increase understanding of interactions between several components of a biological system, is possible only through mathematical representation of the processes involved as a series of nonlinear differential equations (Thornley & Johnson, 1990). The explicit representation of protozoal metabolism within mathematical models of rumen fermentation has received only limited attention (Dijkstra *et al.* 1990).

The present paper describes a mathematical model which simulates the dynamics of ingested nutrients and of microbes within the rumen of cattle, with specific emphasis on the role of the rumen protozoa. The model is a development of the rumen model of Dijkstra *et al.* (1992), such that in addition to bacteria, the protozoa have been included as a state variable. This explicit representation allows evaluation of the interactions of protozoa with bacteria and dietary characteristics. The principal objective of the model is to evaluate concepts and data in order to provide a quantitative understanding of the protozoal dynamics and of the integration of protozoal functioning with other microorganisms and diet composition.

THE MODEL

Principal fluxes for grouped nutrients in the model are shown in Figure 1. The equations that constitute the model, as well as the nomenclature associated with the equations, are listed in the Appendix and generally, notation will follow the lines described by Gill *et al.* (1989).

The model is modified from the dynamic, deterministic rumen model described by Dijkstra *et al.* (1992). Neal *et al.* (1992) evaluated this model and concluded from results of sensitivity analyses and of evaluation against a wide range of dietary inputs, that microbial recycling through the activities of the rumen protozoa had to be represented more accurately. In that model, protozoa are represented as a fixed part of the amylolytic microbial pool, consisting of both bacteria and protozoa, and many rate constants in equations describing protozoal activities are assigned the

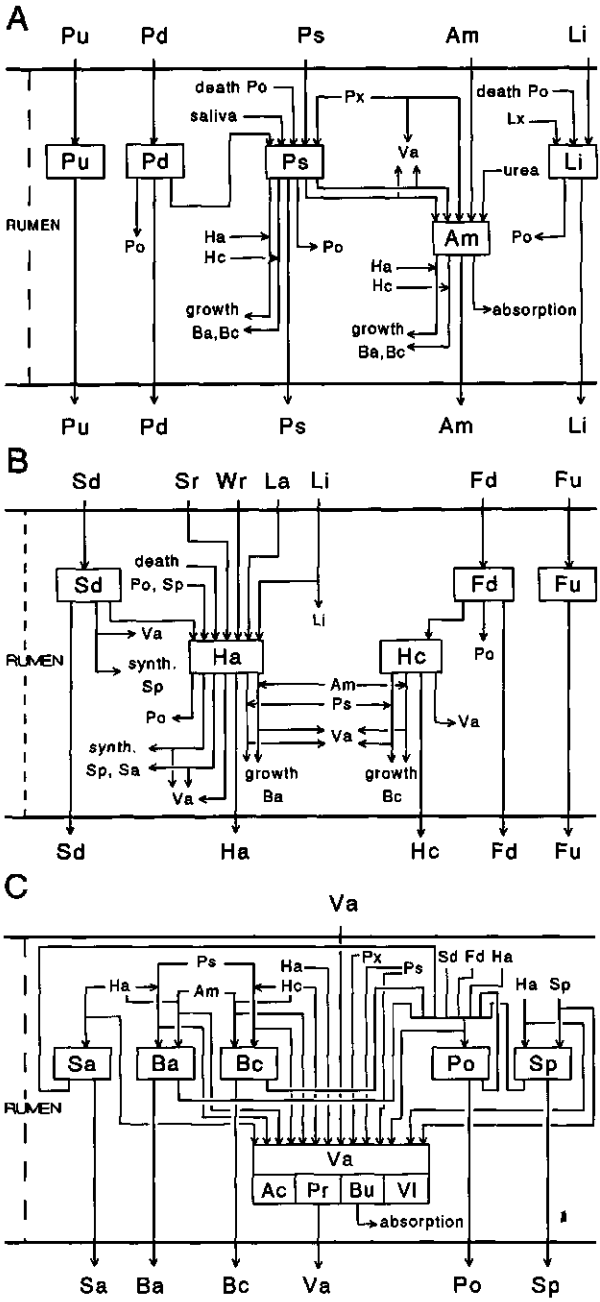

Figure 1. Diagrammatic representation of rumen model with respect to input and output of *protein, ammonia and lipid* (A), *fibre, starch and hexose* (B), and microbial matter and volatile fatty acids (C). Boxes enclosed by solid lines indicate state variables and arrows indicate fluxes. See Appendix for key to abbreviations.

Table 1. *Parameter values.*

Transaction	$M_{i,jk}$ or $M^*_{i,jk}$	T^*_i	v^*_{jk}	$M_{Ha,jk}$ or $M_{Hc,jk}$	$M_{pH,jk}$	$J_{Am,jk}$	$J_{Ps,jk}$	$J_{Ha,jk}$ or $J_{Hc,jk}$	$J_{pH,jk}$	$\Theta_{pH,jk}$	$J_{Sp,jk}$	$\Theta_{Sp,jk}$ or $\Theta_{Va,jk}$
Ac,AcAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48	-	-
Am,AmAb	0.0132	-	1.10	-	7.5	-	-	-	-	7.85	-	-
Am,AmBa	0.00135	-	0.0528	0.0159	-	-	-	-	-	-	-	-
Am,AmBc	0.00135	-	0.0528	0.0159	-	-	-	-	-	-	-	-
Am,UeAm	-	-	0.00165	-	-	0.00621	-	-	-	-	-	-
Bx,BxPo	34.694	-	15.439	-	-	-	-	-	-	-	0.20	1.5
Bu,BuAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48	-	-
Fd,FdHc	0.353	0.83	0.1646	-	5.97	-	-	-	-	22.9	-	-
Fd,FdPo	0.0083	0.83	0.02	-	-	-	-	-	-	-	0.20	1.5
Ha,HaSa	0.0268	-	0.053	-	-	-	-	-	-	-	-	-
Ha,HaVa	0.045	-	0.1646	-	-	0.00861	0.01465	-	-	-	-	-
Ha,HaPo	0.037	-	0.058	-	-	-	-	-	-	-	-	-
Ha,HaSp	0.075	-	0.022	-	-	-	-	-	-	-	0.40	10.0
Hc,HcVa	0.045	-	0.1646	-	-	0.00861	0.01465	-	-	-	-	-
Li,LiPo	0.01	-	0.00075	-	-	-	-	-	-	-	-	-
Pd,PdPs	0.281	0.66	0.0576	-	-	-	-	-	-	-	-	-
Pd,PdPo	0.046	0.66	0.04	-	-	-	-	-	-	-	0.20	1.5
Po,PoPo	34.694	-	15.439	-	-	-	-	-	-	-	0.20	1.5
Pr,PrAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48	-	-
Ps,PsAm	0.0289	-	0.0144	-	-	-	-	0.0165	-	-	-	-
Ps,PsBa	0.0224	-	0.0576	0.0248	-	-	-	-	-	-	-	-
Ps,PsBc	0.0224	-	0.0576	0.0248	-	-	-	-	-	-	-	-
Ps,PsPo	0.04	-	0.04	-	-	-	-	-	-	-	-	-
Px,PxAm	0.02	1.0	0.0144	-	-	-	-	-	-	-	-	-
Sd,SdHa	0.442	1.04	0.2179	-	-	-	-	-	-	-	-	-
Sd,SdPo	0.208	1.04	0.036	-	-	-	-	-	-	-	-	-
Sd,SdSp	0.156	1.04	0.022	-	-	-	-	-	-	-	0.40	10.0
Va,PoDe	0.0054	0.1	4.1	-	-	-	-	-	-	-	-	4.3
Vl,VlAb	0.338	-	7.86	-	-	-	-	-	6.45	6.48	-	-

See Appendix for explanation of notation.

same values as for bacteria. In view of the objective of the present model, a lower degree of aggregation is appropriate (Gill *et al.* 1989). Thus, bacteria and protozoa were represented in separate pools and the parameters in equations related to protozoal metabolic activity were assigned values derived from relevant literature, where available. General protozoal characteristics, which differ from bacterial characteristics, and have been represented in the present model are: utilization of starch and soluble sugars as well as fibre as major sources of carbon, with preference for the former; engulfment of starch to form storage polysaccharides; no utilization of ammonia to synthesize amino acids *de novo*; direct incorporation of dietary or microbial fatty acids to meet lipid formation requirements; preference for insoluble to soluble protein as nitrogen source; engulfment and digestion of bacteria and protozoa; relatively low maximum growth rates; selective retention within the rumen; death and subsequent lysis related to nutrient availability. Justification for the various assumptions associated with this representation of protozoal characteristics is presented in the relevant sections below.

The model comprises nineteen state variables, each representing a pool size in the rumen. Five state variables relate to carbohydrate entities in the rumen: rumen degradable (Fd) and undegradable neutral detergent fibre (Fu), rumen degradable, insoluble starch (Sd), and hexose derived from structural (Hc) and non-structural carbohydrates (Ha). Four state variables relate to nitrogen (Ni) containing entities: rumen undegradable protein (Pu), rumen degradable, insoluble (Pd) and soluble protein (Ps), and ammonia (Am). Five state variables relate to fatty acid containing entities: lipid (Li), and acetic (Ac), propionic (Pr), butyric (Bu), and valeric acid (Vl). Finally, five state variables relate to microbial entities: amylolytic (Ba) and cellulolytic bacteria (Bc), protozoa (Po), amylolytic bacterial (Sa) and protozoal (Sp) storage polysaccharides. In the model, Ha and Hc are the products (mono- or disaccharides) from hydrolysis of non-structural carbohydrates (starch and soluble sugars), lactic acid and glycerol from lipid hydrolysis, and from hydrolysis of structural carbohydrates (cellulose and hemicellulose), respectively. Within the objective of the model, nutrients other than carbon or nitrogen substrates are assumed to be present in non-limiting amounts and consequently these nutrients have not been represented. All pools are expressed in moles except for the microbial pools, which are in grams (g). Relative molecular masses of monomers of protein and carbohydrates and of lipid are assumed to be 110, 162 and 675 g. Volume is expressed in litres (l) and time in days (d). The majority of the transaction kinetics is described using standard expressions from enzyme kinetics (Michaelis-Menten equations); the remainder are described by mass-action forms. Parameters of the Michaelis-Menten equations are given in Table 1; yield, requirement and fraction parameters in Table 2 and microbial growth requirements in Table 3. Michaelis-Menten parameters were often assigned values on a priority basis, given relevant experimental observations. Justification for the various assumptions on parameter values is given in the sections below.

PARAMETERIZATION

Input parameters

The nutrient inputs to the model from the feed are derived from the amount of feed fed to the animal and the chemical composition of the diet. These inputs did not change with progress of time, thus simulating rumen fermentation processes during the day in a frequently fed animal. Estimates of solubility, degradability and digestion turnover times can be determined by the nylon-bag technique, using sources as described by Dijkstra *et al.* (1992). Fractional outflow rates of fluid (k_{FlEx}) and solid phase (k_{SoEx}) are input parameters and are derived from the relevant experiments or multiple regression equations (Owens & Goetsch, 1986). Finally, the pH value of the fluid, time below critical pH and minimum pH reached have to be specified.

Rate constants

Microbial pools, general

As described previously (Dijkstra *et al.* 1992), amylolytic bacteria (Ba) and cellulolytic bacteria (Bc) utilize hexose derived from non-structural carbohydrates and structural carbohydrates, respectively. Protozoa (Po) are assumed to utilize both sources of carbon hexose. Microbial storage polysaccharides are represented in separate pools to accommodate for variation in microbial composition and are linked to utilization of non-structural carbohydrates (Dijkstra *et al.* 1990). On a range of diets, Czerkawski (1976) concluded that the composition of polysaccharide-free DM did not differ to a great extent between bacteria and protozoa. Hence, the polysaccharide-free microbial matter of all groups is assumed to be equal and is given in Table 3 (taken from Dijkstra *et al.* 1992). For simplicity, the predictable difference in cell wall content between bacteria and protozoa was ignored. The estimated amounts of hexose, protein and ammonia required for biosynthesis of bacterial cells were adopted from Dijkstra *et al.* (1992), based on Forrest & Walker (1971) and Stouthamer (1973). The required amounts for biosynthesis of protozoal cells (Table 3) were calculated using the same sources and on the assumptions that, firstly, lipid requirements are met by direct incorporation of dietary and/or microbial long chain fatty acids (Demeyer *et al.* 1978; Williams, 1986), and secondly, that the rate of protozoal amino acid formation from sources other than preformed amino acids is quantitatively unimportant (Coleman, 1986).

Degradable, insoluble protein pool, Pd

There is one input to this pool, from the feed (Eq. 1.2). Three outputs have been represented; hydrolysis of Pd by bacterial proteolytic enzymes (Eq. 1.3), uptake of Pd by protozoa (Eq. 1.4), and outflow from the rumen with the solid phase (Eq. 1.5). Parameters used to calculate Pd hydrolysis (Eq. 1.7 and 1.8) are adopted from Dijkstra *et al.* (1992). In contrast with protozoal uptake of soluble material, which does not seem to be inhibited in presence of storage material within the protozoal cell

(Coleman, 1967; Williams, 1989), protozoal uptake of insoluble material in presence of Sp is assumed to be inhibited to the same extent as uptake of bacteria, explained in the Ba pool section. The maximum uptake rates of protein (v^*_{PdPo} and v^*_{PsPo}) are calculated using the approach described by Dijkstra *et al.* (1992). The maximum utilization of protein for growth can be calculated from the requirement of protein per unit protozoal growth and the maximum growth rate (4.1 /d, described in the Sd section). The maximum fermentation rate of protein by protozoa was assumed to be equal to that of bacteria, since on the one hand, extracts of protozoa were more

Table 2. *Yield, requirement and fraction parameters*

Transaction	$Y_{i,jk}$	$R_{i,jk}$	$f_{j,k}$ or $f_{i,jk}$	Transaction	$Y_{i,jk}$	$R_{i,jk}$	$f_{j,k}$ or $f_{i,jk}$
Ac,InAc	0.0167	-	-	Li,InLi	0.0013	-	-
Am,InAm	0.0588	-	-	Li,PoDe	0.00018	-	-
Am,PsAm	1.257	-	-	Lx,XxPo	-	0.00018	-
Am,PxAm	1.257	-	-	Lx,BaPo	0.00018	-	-
Am,UeAm	2.0	-	-	Lx,BcPo	0.00018	-	-
Ba,AmBa	118.91	-	-	Lx,PoPo	0.00018	-	-
Ba,PsBa	149.48	-	-	Pd,InPd	0.0091	-	-
Bc,AmBc	118.91	-	-	Po,XxPo	142.05	-	-
Bc,PsBc	149.48	-	-	Pr,InPr	0.0135	-	-
Bu,InBu	0.0114	-	-	Ps,InPs	0.0091	-	-
Fd,InFd	0.0062	-	-	Ps,PdPs	1.0	-	-
Fu,InFu	0.0062	-	-	Ps,PoDe	0.0067	-	-
Ha,AmBa	-	1.793	-	Ps,SlPs	0.0010	-	-
Ha,InLi	0.00074	-	-	Px,BaPo	0.0067	-	-
Ha,LaHa	0.0025	-	-	Px,BcPo	0.0067	-	-
Ha,PoDe	0.00011	-	-	Px,PoPo	0.0067	-	-
Ha,PsBa	-	1.291	-	Px,XxPo	-	0.0067	-
Ha,SdHa	1.0	-	-	Pu,InPu	0.0091	-	-
Ha,SpDe	0.0062	-	-	Sa,HaSa	112.5	-	-
Ha,SrHa	0.0062	-	-	Sd,InSd	0.0062	-	-
Ha,WrHa	0.0062	-	-	Sf,SdSp	-	-	0.306
Hc,AmBc	-	1.793	-	Sp,HaSp	112.5	-	-
Hc,FdHc	1.0	-	-	Sp,SdSp	112.5	-	-
Hc,PsBc	-	1.291	-	Vl,InVl	0.0098	-	-
Hf,AmBa	-	-	0.526	Xf,XxPo	-	-	0.885
Hf,AmBc	-	-	0.526	Xx,BaPo	0.00011	-	-
Hf,HaSa	-	-	0.306	Xx,BcPo	0.00011	-	-
Hf,HaSp	-	-	0.306	Xx,PoPo	0.00011	-	-
Hf,PsBa	-	-	0.711	Xx,SaPo	0.0062	-	-
Hf,PsBc	-	-	0.711	Xx,SpPo	0.0062	-	-
-,LcLe	-	-	2.250	Xx,XxPm	-	0.0085	-

See Appendix for explanation of notation.

active than extracts from bacteria in deaminating amino acids (Hino & Russell, 1985), but on the other hand, intact protozoa were less active than bacteria in producing ammonia from a range of proteins (Hino & Russell, 1987). In view of preference of protozoa for insoluble compared with soluble protein and the higher activity against the former (Forsberg *et al.* 1984; Wallace & Cotta, 1988), the high protozoal activity against insoluble protein compared with bacterial activity (Nolan, 1989), and in absence of adequate quantitative data, $M^{*}_{Pd,PdPo}$ was set arbitrarily at 1/6 of bacterial affinity constants for protein hydrolysis, while $M_{Ps,PsPo}$ was set arbitrarily at nearly twice the average value of bacterial affinity constants for uptake of Ps for growth and for fermentation to Am.

Soluble protein pool, Ps

There are five inputs to the Ps pool; from the feed (Eq. 2.2), from saliva (Eq. 2.3), from hydrolysis of Pd (Eq. 2.4), from engulfed microbial and feed protein released into rumen fluid (Eq. 2.5), and from death and lysis of protozoa (Eq. 2.6). Salivation rate is assumed to be related to DM intake and NDF content of the diet (Dijkstra *et al.* 1992). It is assumed that all protein taken up by protozoa (bacterial, protozoal, insoluble degradable, and soluble protein, Eq. 2.15) can be incorporated into protozoal protein. As rationalized by Coleman (1986), protozoa will not utilize the protein from the various sources for growth unless sufficient energy to support growth is available. Thus, a part of engulfed protein (Px) is incorporated into protozoal protein, and the remainder is then partitioned between fermentation and release into rumen fluid (Eq. 2.5), represented in a balance equation. It is assumed that all released protein is soluble. Michaelis-Menten parameters for fermentation of engulfed protein are given in the Am pool section.

Six outputs from the Ps pool are represented; fermentation of Ps by bacterial activity (Eq. 2.7 and 2.8), incorporation of Ps in bacterial biomass (Eq. 2.9 and 2.10), uptake of Ps by protozoa (Eq. 2.11) and outflow of Ps with the fluid (Eq. 2.12). Michaelis-Menten constants for bacterial activity against Ps (Eq. 2.7 to 2.10 and 2.16 to 2.19) are adopted from Dijkstra *et al.* (1992); those for uptake of Ps by protozoa have been explained in the previous section.

Undegradable protein pool, Pu

The Pu pool receives input from the feed only (Eq. 3.2) and outflow from the rumen is with the solid phase (Eq. 3.3).

Ammonia pool, Am

There are five inputs to this pool; from the feed (Eq. 4.2), urea transfer to the rumen (Eq. 4.3; Eq. 4.12 adopted from Dijkstra *et al.* 1992), fermentation of Ps (Eq. 4.4 and 4.5), and fermentation of engulfed protein (Eq. 4.6). As explained in one of the previous sections, the maximum rate of fermentation of protein by protozoa is assumed to be the same as for bacteria. The affinity constant $M_{Px,PxAm}$ (Eq. 4.14)

Table 3. *Composition of polysaccharide-free microbial matter and amount of nutrients required for biosynthesis of bacterial and protozoal cell components: hexose for energy (Hx_{ATP}), hexose incorporated (Hx_{inc}), amino acids incorporated (Aa_{inc}) and ammonia incorporated (Am_{inc})*

Microbial cell component	Polysaccharide free dry weight	Biosynthesis from hexose and amino acids, bacteria			Biosynthesis from hexose and ammonia, bacteria			Biosynthesis from hexose and amino acids, protozoa		
		Hx_{ATP}	Hx_{inc}	Aa_{inc}	Hx_{ATP}	Hx_{inc}	Am_{inc}	Hx_{ATP}	Hx_{inc}	Aa_{inc}
	g/100 g	*mmol/g*			*mmol/g*			*mmol/g*		
Protein	53.0	5.35	-	4.81	5.72	4.08	6.07	5.35	-	4.81
DNA	3.4	0.16	0.15	0.37	0.57	0.23	0.46	0.16	0.15	0.37
RNA	12.3	0.45	0.51	1.22	1.48	0.77	1.54	0.45	0.51	1.22
Lipid	14.3	0.05	1.80	-	0.05	1.80	-	0.14	0.11	-
Cell wall	4.2	0.13	0.04	0.29	0.11	0.27	0.34	0.13	0.04	0.29
Ash	12.9	-	-	-	-	-	-	-	-	-
Total	100	6.14	2.50	6.69	7.93	7.15	8.41	6.23	0.81	6.69
Polysaccharide	-	2.72	6.17	-	2.72	6.17	-	2.72	6.17	-

See Appendix for explanation of notation.

was calculated at the reference diet (Firkins *et al.* 1987), used to parameterize several aspects of the model. $M_{Px,PxAm}$ was set at 0.02 mol Px/g Po, to partition approximately 50% of the protein, remaining after protozoal growth requirements have been met, to ammonia, and to release the other 50% into rumen fluid. The reference turnover time of engulfed protein was set arbitrarily at 1.0 /d.

There are four outputs from the Am pool; utilization of Am by bacteria (Eq. 4.7 and 4.8), absorption through the rumen wall (Eq. 4.9; adopted from Dijkstra *et al.* 1992), and outflow with the fluid (Eq. 4.10). In contrast with bacteria, most protozoa cannot use urea or ammonia to synthesize amino acids (Coleman, 1986; Jouany *et al.* 1988).

Lipid pool, Li

There are three equations representing inputs to this pool; from the feed (Eq. 5.2), lysis of protozoa (Eq. 5.3), and release of engulfed lipid (Lx) not utilized for protozoal growth (Eq. 5.4). The yield of Li from the feed ($Y_{Li,InLi}$), from lysis of protozoa ($Y_{Li,PoDe}$) and from engulfed microbial matter ($Y_{Li,BaPo}$, $Y_{Li,BcPo}$ and $Y_{Li,PoPo}$) is calculated on the assumption that lipid is completely hydrolysed to long chain fatty acids and glycerol (molecular weight glycerol = 92 g). The amount of engulfed Li released into rumen fluid is represented in a balance equation, assuming that all engulfed Li not utilized for growth is released again. There are two outputs from the pool; uptake of Li by protozoa (Eq. 5.5) and outflow with the solid phase (Eq. 5.6). The maximum rate of lipid uptake v^*_{LiPo} (= 0.00075 mol Li/(g Po·d)) was calculated from maximum rate of protozoal growth and the chemical composition (Table 3). The affinity constant $M_{Li,LiPo}$ (= 0.01 mol/l) was calculated to partition sufficient lipid to meet protozoal growth requirements at low lipid contents of the diet. It should be noted that high lipid concentrations in the diet are toxic to protozoa and cause a reduction or elimination of protozoa in the rumen (Jouany *et al.* 1988). In absence of quantitative data, this aspect has been ignored and it is assumed that the lipid concentration in simulated diets is at a level too low to affect protozoa deleteriously.

Degradable starch pool, Sd

There is one input to the Sd pool; from the feed (Eq. 6.2), and four outputs; hydrolysis to amylolytic hexose (Eq. 6.3; with parameters adopted from Dijkstra *et al.* 1992), engulfment of starch for protozoal maintenance and growth (Eq. 6.4) and storage polysaccharide formation (Eq. 6.5), and outflow from the rumen with the solid material (Eq. 6.6). Maximum velocity of starch engulfment is calculated from uptake rates of carbohydrates to achieve maximum protozoal growth rate and Sp formation. Values of maximum growth rates reported in literature for smaller protozoa, which primarily utilize starch and sugars but no fibre, and larger protozoa, which utilize both fibre and starch and sugars, are 4.1 and 1.7 /d, respectively (review Williams & Coleman, 1988). Application of the Pirt double reciprocal equation of growth yield vs. dilution rate (Pirt, 1965) and protozoal maintenance requirement (described in the Po pool section) yields a maximum uptake rate of starch or sugars

and of fibre for maintenance and growth of 0.036 and 0.020 mol/(g Po·d), respectively. On the assumption that maximum content of storage polysaccharides in protozoal DM equals 40% (Czerkawski, 1976), maximum uptake rates of starch and sugars v^*_{SdSp} and v^*_{HaSp} to form storage polysaccharides are 0.022 mol/(g Po·d). In absence of quantitative information to estimate protozoal affinity constants of Sd and Ha, these were arbitrarily assigned values given in Table 1. Some justification for these values are the observations that firstly, several protozoal species do not synthesize Sp from soluble sugars (review Coleman, 1986) and consequently, $M_{Ha,HaPo}$ has been set at a lower value than $M_{Ha,HaSp}$; and secondly, starch can be stored within the protozoal cell and fermented several hours later, and consequently, $M_{Sd,SdPo}$ has been set at a higher value than $M_{Sd,SdSp}$. The utilization of Sd and Ha for Sp synthesis is inhibited sigmoidally by Sp content of the protozoal biomass (Eq. 6.12 and 9.20), since starch is engulfed rapidly when protozoa have been starved for a certain period, but starch uptake declines when protozoa are completely filled with starch particles (Coleman, 1986), and since on soluble sugar substrates, Sp content is not increased above a certain maximum level even if soluble sugar concentration in the fluid is increased further (Van Hoven & Prins, 1977). Thus, at Sp contents higher than 40% of total protozoal biomass, synthesis of Sp from Sd and Ha is assumed to be completely inhibited and a sigmoidal response equation, giving a sharp switch-off characteristic (Gill *et al.* 1989) is required, with inhibition constants $J_{Sp,HaSp}$ and $J_{Sp,SdSp}$ set at 0.4 g Sp/(g (Sp + Po)) and steepness parameters $\Theta_{Sp,HaSp}$ and $\Theta_{Sp,SdSp}$ set at 10.

Degradable fibre pool, Fd

Feed input to the Fd pool has been represented (Eq. 7.2). There are three outputs from the pool: to cellulolytic hexose (Eq. 7.3; hydrolysis parameters from Dijkstra *et al.* 1992), to protozoa (Eq. 7.4) and outflow from the rumen with the solid material (Eq. 7.5). The maximum uptake rate of Fd by protozoa and inhibition related to relative amounts of Sp (Eq. 7.9) has been explained in previous sections. The affinity constant $M^*_{Fd,FdPo}$ was set arbitrarily. In contrast with bacteria, Fd utilization by protozoa is not assumed to be inhibited directly by low pH values. Rather, an indirect effect as a result of high availability of (rapidly degradable) substrates, resulting in high protozoal death rates (Eq. 14.10 and 14.11; see protozoa pool section), has been included.

Undegradable fibre pool, Fu

This pool receives input from the feed (Eq. 8.2) and outflow is with the solid phase (Eq. 8.3).

Amylolytic hexose pool, Ha

There are seven inputs to the pool; four of these inputs are directly from the feed, viz. water soluble carbohydrates (Eq. 9.2), soluble starch (Eq. 9.3), lactic acid (Eq. 9.4)

and glycerol, arising from hydrolysis of dietary lipid (Eq. 9.5). Other inputs are from starch hydrolysis (Eq. 9.6) and hydrolysis of protozoal Li (Eq. 9.7) and Sp (Eq. 9.8) released by death and lysis of protozoa, with parameters described before and in the Po pool section. The yield factors of hexose derived from lipid are calculated on the assumption that 1 mol of lipid yields 0.5 mol hexose (from the glycerol part of lipid).

There are seven outputs from the pool; utilization of Ha for bacterial growth with Am (Eq. 9.9) or Ps (Eq. 9.10), utilization for Sa formation (Eq. 9.11), utilization for bacterial functions not related to growth (Eq. 9.12), uptake for protozoal maintenance and growth (Eq. 9.13) and Sp formation (Eq. 9.14), and washout from the rumen (Eq. 9.15). Parameters in equations related to bacterial activity were taken from Dijkstra *et al.* (1992). Protozoal cells do not control soluble sugar uptake rate (Williams, 1979) and the Sp content of protozoal cells does not inhibit glucose uptake rate (Williams, 1989). Thus, the utilization of Ha by protozoa (Eq. 9.13) has been represented as an overall uptake rate, of which the amount utilized for Sp synthesis is subtracted while the remainder is utilized for maintenance and growth. This representation will rapidly increase death rate of protozoa at Ha concentration levels above the level supporting maximum Sp formation, thus simulating the observed lysis of holotrich protozoa in presence of excess substrate (review Williams & Coleman, 1988). Calculation of the rate constants in Eq. 9.13 and 9.14 has been described in the Sd pool section.

Cellulolytic hexose pool, Hc

There is one input to this pool; from Fd hydrolysis (Eq. 10.2), and four outputs, representing growth with Am (Eq. 10.3) and Ps as nitrogen source (Eq. 10.4), utilization for bacterial non-growth related functions (Eq. 10.5) and washout form the rumen (Eq. 10.6). As before, parameters have been calculated by Dijkstra *et al.* (1992).

Amylolytic bacterial pool, Ba

There are two inputs to the amylolytic bacterial pool, related to the nitrogen sources used for growth: Am (Eq. 11.2) or Ps (Eq. 11.3), with the yield factors calculated from the chemical composition. Two outputs from the Ba pool are represented: engulfment of bacteria by protozoa (Eq. 11.4), and washout from the rumen with the fluid (Eq. 11.5), since amylolytic bacteria are assumed to live free in rumen fluid. Although selective engulfment of bacteria has been reported, there is no consistent pattern between protozoal species (review Coleman, 1989). Thus, in the model it is assumed that bacteria (the pools Ba, Bc and Sa) are engulfed in the proportion in which they are present (Eq. 11.7). Two of the parameters representing engulfment (v^*_{BxPo} and $M_{Bx,BxPo}$) have been calculated by Dijkstra *et al.* (1992), based on engulfment rate data presented by Coleman & Sandford (1979). In addition, the inhibitory effect of protozoal storage polysaccharides on engulfment of bacteria (Coleman, 1975) has been included. Observations made by Coleman (1975) with *Entodinium caudatum* indicate that this inhibition can be modelled in a sigmoidal way:

firstly, in protozoa filled with relative small amounts of starch, bacterial uptake rate was not limited; secondly, engulfment of bacteria was never completely inhibited, even if protozoa appeared to be completely filled with starch. The inhibition constant $J_{Sp,BxPo}$ (= 0.2 g Sp/(g Sp + Po)) and steepness parameter $\Theta_{Sp,BxPo}$ (= 1.5) were set arbitrarily to give weak sigmoidal response and half-maximum rate achieved at assumed half-maximum amount of Sp.

Amylolytic bacterial storage polysaccharides pool, Sa

There is only one input to this pool; from the utilization of Ha (eq. 12.2), with rate constants as described in the Ha section. The two outputs from the Sa pool (Eq. 12.3 and 12.4) are equivalent to the outputs from the Ba pool (Eq. 11.4 and 11.5).

Cellulolytic bacterial pool, Bc

As with Ba, there are two inputs to this pool, representing growth with hexose and either Am (Eq. 13.2) or Ps (Eq. 13.3) as nitrogen source, and two outputs from the pool, representing engulfment by protozoa (Eq. 13.4) and washout from the rumen (Eq. 13.5). In contrast with Ba, Bc pass out of the rumen at a rate equal to the solid phase outflow rate (k_{SoEx}) (Cheng & Costerton, 1980).

Protozoal pool, Po

There is one input to this pool, from the utilization of carbohydrates taken up and not utilized for protozoal maintenance (Eq. 14.2). In contrast with bacteria, the maintenance requirement of protozoa ($R_{Xx,XxPm}$) is assumed not to change with nitrogen availability, in view of the generally high availability of nitrogen sources for protein synthesis. However, if the availability of engulfed protein (Px) to support protozoal growth is lower than the corresponding availability of energy, the production of protozoa is calculated according to the Px availability (Eq. 14.2) and the remaining carbohydrates are fermented completely (Eq. 16.42). In absence of data on maintenance requirements of protozoa, this value has been set arbitrarily at 0.0085 mol/(g Po·d). General hexose entities taken up are: degradable fibre and starch, amylolytic hexose, engulfed microbial polysaccharides and hexose arising from the glycerol moiety of engulfed microbial lipid (Eq. 14.7). The utilization rates of the hexose entities have been described in the relevant sections. There are three outputs from the pool; engulfment by other protozoa (Eq. 14.3), death of protozoa (Eq. 14.4) and washout of protozoa from the rumen (Eq. 14.5). Uptake of small protozoa by larger ones has been reported (e.g. review Coleman, 1986); however, in absence of quantitative data, rate constants in Eq. 14.3 and 14.9 were assumed to be the same as for engulfment of bacteria. Protozoa have been observed to degenerate and burst, particularly on diets rich in easily degradable carbohydrates (review Williams & Coleman, 1988). The cause of protozoal lysis is probably the inability of protozoa to control soluble substrate entry (Williams, 1979) and the subsequent intracellular buildup of acidic fermentation products (Prins & Van Hoven, 1977). Thus, in the

model, the amount of VFA produced from fermentation of hexose entities per unit of time and protozoal biomass (Eq. 14.10 - 14.12) will determine the death rate of protozoa. A sigmoidal response was assumed to obtain low death rates at low nutrient availabilities with a rapid increase when hexose entities taken up are increased. The value of maximum death rate was assumed to be the same as the maximum growth rate of protozoa (see Sd section), while the affinity and steepness parameter were set arbitrarily. Protozoa are selectively retained within the rumen (review Jouany *et al.* 1988) and outflow rate of protozoa is set at 45% of k_{SoEx} (Eq. 14.13) (data summarized by Faichney, 1989).

Protozoal storage polysaccharides pool, Sp

There are two inputs to this pool, representing synthesis from Ha (Eq. 15.2) and Sd (Eq. 15.3), with utilization rates as explained in the relevant sections. The three outputs from this pool (Eq. 15.4 to 15.6) are equivalent to the outputs of the protozoal pool (Eq. 14.3 to 14.5).

Volatile fatty acid pools, Ac, Pr, Bu and Vl

The inputs to and outputs from the Va pools (Ac, Pr, Bu and Vl) only differ in stoichiometric yield factors applied and are considered together in this section. There are fourteen inputs to and two outputs from each of these pools. Inputs are from the feed, from fermentation of Ha and Hc related to bacterial growth with Am and Ps or related to bacterial non-growth related functions, from fermentation of Ha related to Sd formation, from fermentation of Ps by bacteria and Px by protozoa, from fermentation of Fd, Sp and Ha related to protozoal maintenance and growth, and from fermentation of Ha and Sd related to Sp formation. The hexose fermented as a fraction of total hexose spend in a reaction can be calculated from Table 3. Stoichiometric yield parameters are given by Murphy *et al.* (1982) for roughage and concentrate diets, and intermediate values are used in the model for diets of which the DM roughage content is 40 to 60%. In absence of quantitative data, stoichiometric yield parameters for lactic acid and glycerol fermentation are equal to those for water soluble carbohydrate fermentation, and cellulose and hemicellulose are assumed to behave as one nutritional entity. Although it is recognized that rumen protozoa hardly produce propionic acid, but rather acetic and butyric acid (review Jouany *et al.* 1988), stoichiometric parameters as calculated by Murphy *et al.* (1982) do not account for differences in microbial populations in production of Va. Besides, it is assumed that the type of Va formed does not affect other rumen fermentation characteristics or the interactions between protozoa, bacteria and dietary factors and thus, the differences in endproducts produced by different microbial species on the same substrate has been ignored.

The outputs from each Va pool are absorption through the rumen wall, with parameters adopted from Dijkstra *et al.* (1992), and outflow with the rumen fluid.

Model summary

The model is completely defined by Eq. 1.1 to 19.35 in the Appendix. The differential equations of the nineteen state variables are integrated numerically for given initial conditions and parameter values in Tables 1 and 2. The computer program was written in the simulation language ACSL (Advanced Continuous Simulation Language, Mitchell & Gauthier, 1981) and run on a VAX computer. Integration interval of $\Delta t = 0.01$ was used, with a fourth-order fixed-step-length Runga-Kutta method and the results presented in the next section were obtained by taking the predictions at 30 d.

RESULTS

The results of model evaluation are presented in 3 parts. Firstly, sensitivity of protozoal dynamics to changes in selected parameter values associated with protozoa was examined. Secondly, published experiments were simulated and results of these simulations were compared with reported values. Finally, response of the model to changes in input variables (diet composition, feed intake level, fluid and solid passage rates) was examined.

Sensitivity analysis

The sensitivity of the model to changes in selected parameters was examined on a high roughage diet (Diet R; 70% grass silage, 20% maize silage and 10% concentrates) and a high concentrate diet (Diet C; 23% grass silage, 7% maize silage and 70% concentrates) at a simulated continuous feed intake of 18 kg DM/d for a dairy cow. The contents of the grass silage were (in g/kg DM): NDF, 525; starch and sugars, 50; N, 28; of the maize silage: NDF, 425; starch and sugars, 300; N, 15; and of the concentrate: NDF, 200; starch and sugars, 420; N, 25. For both diets, fluid passage rate (k_{FlEx}), solid passage rate (k_{SoEx}), and rumen volume (V_{Ru}) were set at 2.7/d, 1.0/d and 90 l, respectively. The pH of rumen fluid for Diet R and C was set at 6.3 and 6.1, respectively. The simulated pattern of nutrients available for absorption is presented in Table 4. In view of the large number of parameters in the model, only a few parameters associated with protozoal dynamics were selected for presentation, either because of limited information to derive the parameter value or a likely large effect on protozoal biomass in the rumen. Selected parameters were set at +/- 50% and +/- 25% of model values.

A decrease in the affinity parameter related to engulfment of bacteria ($M_{Bx,BxPo}$) increased the amount of bacterial matter engulfed and decreased the bacterial organic matter in the rumen (Figure 2). The protozoal biomass in the rumen was hardly affected however, because in the model, it is assumed that a large part of the engulfed microbial matter, which consists mainly of protein and nucleic acids (Table 3), will be excreted in rumen fluid. The lower bacterial biomass in the rumen due to an increase in $M_{Bx,BxPo}$ resulted in decreased degradation of NDF and starch and

Table 4. *Simulated nutrient profile and microbial populations with diets used in sensitivity analyses*

	Diet R	Diet C
Duodenal flow of OM (g/d):		
Starch and soluble sugars	763	1183
NDF	3316	2824
Total NAN	411	428
Microbial NAN	261	205
Protozoal NAN	14	21
Non-microbial NAN	150	223
VFA production (mol/d)	64.9	59.7
VFA molar proportions (mol/100 mol):		
Acetate	69.2	56.4
Propionate	14.4	26.0
Butyrate	10.3	9.1
Valerate	6.1	8.4
In rumen (g):		
Bacterial N	209.5	104.7
Protozoal N	30.0	46.2

Diet R, 90% grass/maize silage, 10% concentrate; Diet C, 30% grass/maize silage, 70% concentrate; NAN, non-ammonia nitrogen; NDF, neutral detergent fibre; VFA, volatile fatty acid.

sugars in the rumen, decreased bacterial NAN outflow but increased non-microbial NAN outflow, so that total NAN flow to the duodenum remained about the same (diet C) or was increased slightly (diet R). Such changes resulted in a marked increase in efficiency of microbial protein synthesis and would significantly affect the profile of nutrients available for absorption. An increase in the inhibition constant for the engulfment of bacteria with respect to the storage polysaccharide content of protozoa ($J_{Sp,BxPo}$) resulted in increased bacterial biomass and efficiency of microbial protein synthesis as well (results not shown).

The maintenance requirement of protozoa ($R_{Xx,XxPm}$) had a very significant effect on protozoal and bacterial biomass in the rumen and hence on degradation and outflow of nutrients (Figure 2). An increase in $R_{Xx,XxPm}$ resulted in decreased growth and biomass of protozoa because more hexose was directed to maintenance. Consequently, the amount of bacterial matter in the rumen increased significantly. Increasing the protozoal maintenance requirement to 150% of the value in Table 1 resulted in simulated extinction of protozoa on both diets, while on diet C a decrease of this requirement to 75% of the model value already resulted in complete disappearance of cellulolytic bacteria. The model appeared to be very sensitive to the affinity constant related to death of protozoa ($M_{Va,PoDe}$) as well. Decreasing this

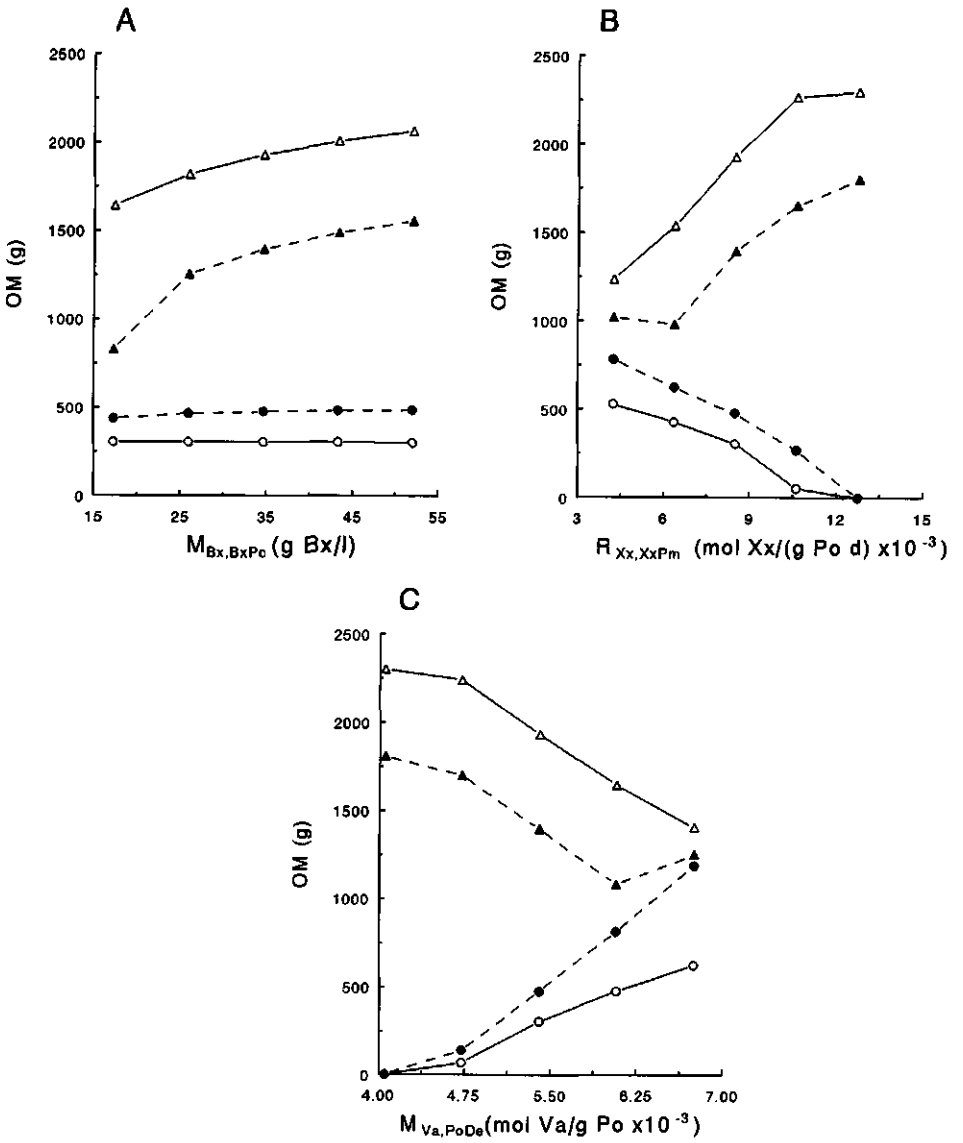

Figure 2. The sensitivity of total microbial OM and of protozoal OM in the rumen to the affinity constant for uptake of bacteria by protozoa ($M_{Bx,BxPo}$; graph A), to the maintenance requirement of protozoa ($R_{Xx,XxPm}$; graph B), and to the affinity constant related to death of protozoa ($M_{Va,PoDe}$; graph C) for the high roughage diet (Diet R) and the high concentrate diet (Diet C). (△), microbial OM for Diet R; (▲), microbial OM for Diet C; (○), protozoal OM for Diet R; (●), protozoal OM for Diet C.

value by 25%, which means that protozoal death rate is increased, resulted in disappearance of protozoa on both diets, while on diet C cellulolytic bacteria disappeared with an increase of this value to 125% of the model value.

Comparison between simulated and experimental values

Values of nutrient supply at different intake and feed N levels reported by Firkins *et al.* (1987) for steers fed 50% hay, 50% concentrate diets were used to set a number of microbial parameters in the model, and the observed values were compared with model simulations (Table 5). As expected, there was a close agreement between observed and simulated duodenal flows of OM. However, as in the previous model (Dijkstra *et al.* 1992), ammonia concentration was not predicted well. The microbial marker used by Firkins *et al.* (1987) did not allow separate estimates of the protozoal and bacterial N flow to the duodenum. Model simulations indicated a slight decrease in the proportion of protozoal N in total microbial N-flow with increased feed intake level (13% and 11% on low and high intake level, respectively) and with increased dietary N level (13% and 11% on low and high feed N level, respectively).

Model simulations on protozoal biomass in the rumen and protozoal turnover and lysis compared with observations obtained by isotope dilution procedures in cattle and sheep, are presented in Tables 6 and 7. In case of sheep, the simulated amount of saliva produced was set at a constant level of 12 l/d. The observed and simulated protozoal turnover is assumed to be due to death and passage of protozoa only, because labelled choline in engulfed protozoa will likely be incorporated again (Leng, 1982). The simulated protozoal pool sizes in cattle and sheep agreed favourably with observed values, except for the pool size observed on the low concentrate diet (Ffoulkes & Leng, 1988). The simulated protozoal contribution to total ruminal microbial N varied from 12% on the oaten/lucerne chaff diet supplemented with untreated soya bean meal (Krebs *et al.* 1989) to 29% on the 70% oaten chaff, 30% molasses diet (Ffoulkes & Leng, 1988). Both observations and simulations indicated a decreased protozoal biomass when N availability was increased (the casein supplemented oaten chaff/lucerne/molasses diet (Leng *et al.* 1984) compared with the unsupplemented diet (Leng, 1982) and the diet containing untreated soya bean meal compared with protected soya bean meal (Krebs *et al.* 1989). The turnover time of protozoa in the rumen was not predicted well, without any apparent relationship of level or trend of the difference between observed and predicted values to dietary composition or pattern of protozoal species present. Although predicted contribution of protozoal lysis to protozoal turnover in the rumen did not always agree with observed values, both predicted and observed values indicated the major contribution of lysis (50-85%) to protozoal turnover.

A number of methods has been used to measure protozoal N flow to the duodenum. In Table 8, results of a comparison between model simulations and observations on steers reported by Cockburn & Williams (1984) are presented. This experiment was chosen because of possibility to compare several marker methods

Table 5. *Comparison of experimentally observed parameters (obs) and parameters predicted by the model (pred) for steers fed diets at low (4.8 kg dry matter/d) and high (7.2 kg dry matter/d) intake levels and at two levels of urea infused (0.4% or 1.2% of diet dry matter)*[1]

	Low		High		0.4%		1.2%	
	obs	pred	obs	pred	obs	pred	obs	pred
Duodenal flow of OM (g/d):								
NDF	1203	1235	2009	2044	1591	1617	1588	1589
Total NAN	107	108	163	165	130	133	140	139
Microbial NAN	62	65	98	96	78	76	83	86
Protozoal NAN	-	8	-	11	-	10	-	9
Non-microbial NAN	45	42	64	69	53	56	57	53
Ammonia concentration (mM)	4.8	4.6	5.3	4.0	3.6	2.7	6.6	6.1
In rumen (g):								
Bacterial N	-	38.0	-	48.9	-	41.7	-	47.4
Protozoal N	-	15.0	-	16.3	-	16.5	-	15.2

NAN, non-ammonia nitrogen; NDF, neutral detergent fibre; VFA, volatile fatty acid.
[1] Observations from Firkins *et al.* (1987).

Table 6. *Comparison of experimentally observed parameters (obs) and parameters predicted by the model (pred) for sheep*

	Diet							
	O		OC		OSU		OSP	
	obs	pred	obs	pred	obs	pred	obs	pred
Rumen pool size of (g):								
Protozoal N	2.4	2.1	1.4	1.6	1.5	1.7	1.6	1.8
Microbial N	-	7.3	-	10.3	-	14.5	-	14.1
Protozoal turnover time (d)	0.83	0.49	0.67	0.84	0.53	1.07	0.32	1.06
Protozoal lysis (%)	63	80	65	64	-	59	-	59

Diet O, oaten chaff/lucerne/molasses (Leng, 1982).
Diet OC, oaten chaff/lucerne/molasses/casein (Leng *et al.* 1982).
Diet OSU, oaten chaff/lucerne chaff/untreated soya bean meal (Krebs *et al.* 1989).
Diet OSP, oaten chaff/lucerne chaff/protected soya bean meal (Krebs *et al.* 1989).

(ribonucleic acid (RNA) as general microbial marker; aminoethylphosphonic acid (AEPA) as protozoal marker; diaminopimelic acid (DAPA) as bacterial marker and the amino acid profile (AA) of protozoa, bacteria and feed to measure all fractions). The simulated duodenal flow of total NAN (which varied from 66 to 111% of dietary N

intake) was always lower than the observed flow (from 94 to 181% of dietary N intake). The simulated percentage of microbial NAN to total NAN flow agreed with the value obtained by the modified AA (mAA) method, but was always lower than values obtained by the RNA or DAPA+AEPA method. As indicated previously, the simulated protozoal contribution to total microbial N in the rumen increased with decreases in N availability, but due to the increased non-microbial NAN flow (treatment TC compared with C and GM) the percentage of protozoal NAN to total NAN flow was not increased. The AEPA+DAPA method clearly overestimated microbial NAN flow, and both simulated protozoal and bacterial NAN percentage flows were lower than values obtained with AEPA and DAPA. Simulated protozoal NAN (which varied between 6 and 11% of total NAN flow) was in the range obtained with the RNA-DAPA method (between -4 and 15%) but lower than the mAA method (between 9 and 25%). An important point in these comparisons is that disagreements between simulated and observed values partially result from inadequacies of input data describing the diets or errors in experimental measurements.

Response to changes in input parameters

Changing the passage rates up to +/- 50% of the standard model values used for both diets (k_{FlEx} = 2.7/d; k_{SoEx} = 1.0/d) had large effects on the simulated amounts of protozoal and bacterial OM in the rumen (Figure 3A and 3B). An increase in either the fluid or solid passage rate decreased total microbial OM in the rumen. In contrast with the continuous decline of protozoal OM in response to increased values of k_{SoEx}, increases in k_{FlEx} at low values caused protozoal OM to increase, because the faster outflow of amylolytic bacteria raised ruminal hexose availability to be used by protozoa. Further increases resulted in reduced protozoal OM because the increased hexose availability caused rapidly increased fractional death rates of protozoa (equations 14.10-14.12).

The effects of varying the NDF, starch, sugars or N content of diets R and C on microbial OM in the rumen, while keeping all other parameters constant, are presented in Figure 4. Increasing the dietary NDF content increased the total microbial OM in the rumen, but did not or only to a minor extent increase ruminal protozoal OM (Figure 4A). The effect of changes in the amount of starch in the diet had a very pronounced effect on protozoal OM (Figure 4B). On diet R, protozoal contribution to total microbial OM increased from 7% when no starch was included up to 17% on the 400 g/kg DM level. However, on diet C, the protozoal biomass was seen to be slightly decreased at such high (> 300 g/kg DM) starch contents. The reason for this reduction is the rapid increase in fractional protozoal death rate (from 0.74 on the lowest to 1.35 /d on the highest starch level). Such elevated death rates were responsible for the decrease in protozoal biomass with increased dietary sugar levels on diet C as well (Figure 4C). However, on diet R, a maximum amount of protozoal OM is reached at dietary sugar levels of approximately 75 g/kg DM. Decreasing the N availability in the rumen by reducing the dietary N content decreased the microbial

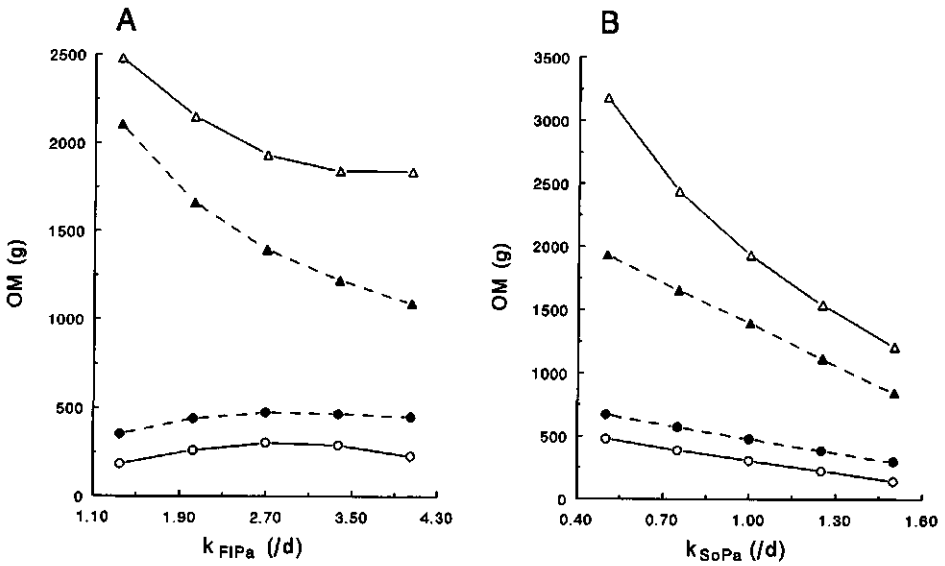

Figure 3. The sensitivity of total microbial OM and of protozoal OM in the rumen to the fractional fluid passage rate (k_{FIEx}; graph A) and solid passage rate (k_{SoEx}; graph B) for the high roughage diet (Diet R) and the high concentrate diet (Diet C). (△), microbial OM for Diet R; (▲), microbial OM for Diet C; (○), protozoal OM for Diet R; (●), protozoal OM for Diet C.

OM in the rumen (Figure 4D). However, even at the lowest N level, engulfment of bacterial and protozoal matter resulted in sufficient availability of protein to support protozoal growth. Thus, the decreased protozoal OM at low dietary N levels could not be attributed to limiting N availability, but rather was the result of high protozoal death rates induced by high energy availability, which in turn was the result of low bacterial growth rates due to the limiting N availability. These relatively low bacterial growth rates at low dietary N levels were the result of a high utilization of hexose for non-growth purposes as shown before (Dijkstra *et al.* 1992).

In Figure 5, the responses of microbial and protozoal OM to changes in feed intake level from 5 to 25 kg DM/d of diets R and C are presented. In order to achieve sensible steady state values, some input values had to be modified (rumen volume, passage rates and pH). These values were changed using a linear regression of DM intake vs rumen volume, fluid and solid passage rate, calculated from the data reported by Robinson *et al.* (1985 and 1987) (feed intake 6.4 to 18.5 and 5.3 to 21 kg DM/d, respectively) and pH according to the latter (regression results not shown). Increasing the feed intake level to approximately 20 kg DM/d increased both protozoal and total microbial OM, but a further increase caused protozoal and microbial OM to decrease. On the lowest feed intake level, protozoal contribution to total microbial

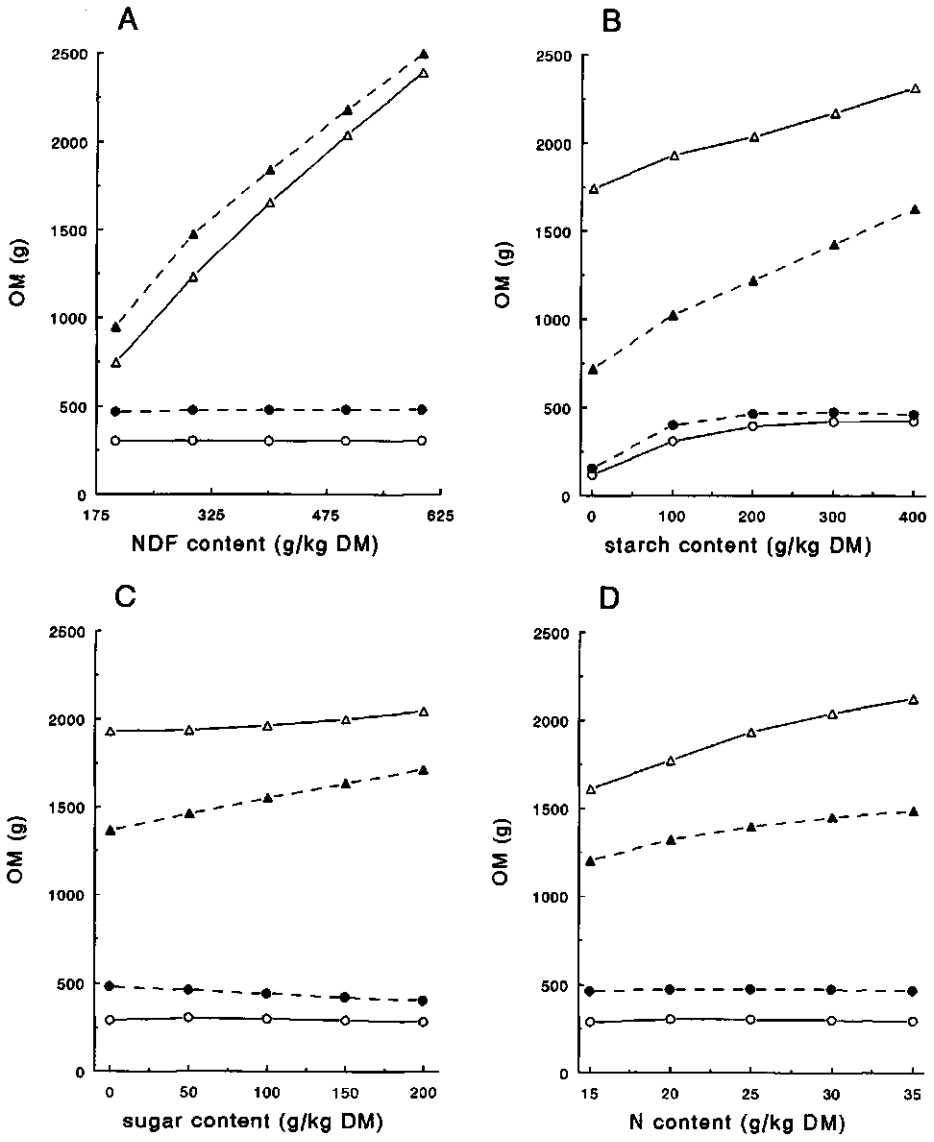

Figure 4. The sensitivity of total microbial OM and of protozoal OM in the rumen to the dietary content level of NDF (graph A), starch (graph B), sugars (graph C) and nitrogen (graph D) for the high roughage diet (Diet R) and the high concentrate diet (Diet C). (△), microbial OM for Diet R; (▲), microbial OM for Diet C; (○), protozoal OM for Diet R; (●), protozoal OM for Diet C.

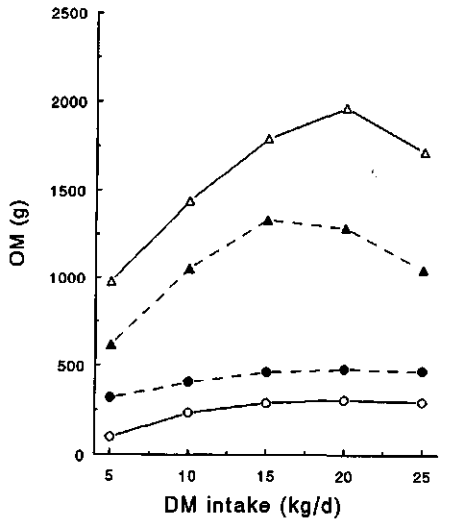

Figure 5. The sensitivity of total microbial OM and of protozoal OM in the rumen to the DM intake level for the high roughage diet (Diet R) and the high concentrate diet (Diet C). (△), microbial OM for Diet R; (▲), microbial OM for Diet C; (○), protozoal OM for Diet R; (●), protozoal OM for Diet C.

OM was 10 and 52% on diet R and C, respectively. However, increasing the feed intake up to 15 kg DM/d increased this contribution to 16% on diet R while on diet C the contribution was decreased to 35%. Further increases increased protozoal contribution to 17 and 45% on diet R and C, respectively. As rumen volume is changed with changes in feed intake level, it is interesting to note the changes in concentration of protozoal OM. On diet R, this concentration rapidly increased from 1.80 g/l at 5 kg DM/d up to 3.6 g/l at 15 kg DM/d, and then decreased to 2.7 g/l at 25 kg DM/d; on diet C, the increase was much less and concentration started to decline at approximately 10 kg DM/d already (5.9, 6.0, 5.7, 5.0 and 4.3 g/l at 5, 10, 15, 20 and 25 kg DM/d, respectively).

DISCUSSION

In several papers, the relative roles of protozoa and bacteria in degradation of carbohydrates and protein, in efficiency of microbial protein synthesis, and ultimately supply of nutrients available for absorption have been pointed out (reviews Veira, 1986; Coleman, 1986; Jouany *et al.* 1988). The basic objèctive of the model was to achieve an integrated understanding of these roles as determined by dietary

Table 7. *Comparison of experimentally observed parameters (obs) and parameters predicted by the model (pred) for cattle*

	Diet					
	HC		LC		RG	
	obs	pred	obs	pred	obs	pred
Rumen pool size of (g):						
Protozoal N	16	14	24	16	15	16
Microbial N	-	55	-	56	-	91
Protozoal turnover time (d)	0.93	0.69	1.07	0.90	1.95	1.26
Protozoal lysis (%)	80	77	68	71	83	54

Diet HC, 30% oaten chaff, 70% molasses (Ffoulkes & Leng, 1988).
Diet LC, 70% oaten chaff, 30% molasses (Ffoulkes & Leng, 1988).
Diet RG, fresh ryegrass (Leng, 1986).

characteristics. A microbial ecosystem, in which several species are growing, is complicated because the state variables (amount of substrates and microbial biomass) are changing not only with respect to time, but with respect to each other as well (Bazin, 1981). In such a complex system, dynamic, mechanistic models provide a way of bringing together knowledge about the state variables, in order to give a coherent view of behaviour of the whole system (Thornley & Johnson, 1990). Reichl & Baldwin (1976) described a linear programming model of rumen fermentation in which eight microbial groups, including protozoa, were represented. However, considerable simplifications of the predicted rumen ecosystem occurred and the authors concluded that additional concepts regarding microbial interactions were required. Bazin (1981) used mathematical equations to illustrate the way interrelationships among microbial species could be represented to obtain realistic predictions of dynamics of multi-species systems. Application of the Michaelis-Menten equations, which are often used to describe microbial kinetics (Pirt, 1975), allowed Dijkstra *et al.* (1992) to represent protozoal predation on bacteria. Although sensitivity analysis of their model indicated in general realistic relationships between protozoa and bacteria (Neal *et al.* 1992), considerable simplifications had to be made, the most obvious one being the representation of a microbial group consisting of both amylolytic bacteria and protozoa. This resulted in incorrect predictions of fibre degradation when starch content of the diet increased or when a defaunated animal was simulated. In the present model, emphasis has been placed on the representation of protozoa and cellulolytic and amylolytic bacteria in separate groups. Important items in analyses of such multi-species systems are the factors which allow populations to coexist and stability of coexistence (Fredrickson, 1977; De Freitas & Fredrickson, 1978). A general condition for stable coexistence is that the number of

nutrients, having rate limiting effects on the competitors, equals or exceeds the number of populations in the system (De Freitas & Fredrickson, 1978). For the present model, in most dietary situations energy substrates would limit growth, while N substrates would affect microbial growth at relatively low N availability only. Hence, in early versions of the model, which did not include protozoal predation on other protozoa (Eq. 14.3) and in which death rate (Eq. 14.4) was fixed, the two substrates limiting growth (amylolytic and cellulolytic hexose) led to exclusion of either cellulolytic and amylolytic bacteria or of protozoa. Thus, although the density of the prey (cellulolytic and amylolytic bacteria) significantly affected the amount of bacterial matter engulfed by protozoa (Eq. 11.4, 12.3 and 13.4), this was not sufficient to allow the three populations to coexist. To obtain biologically realistic coexistence of the populations, in the present model protozoal death rate was related to substrate availability and build-up of fermentation endproducts within protozoa (Eq. 14.10 and 14.12) (Prins & Van Hoven, 1977; Williams, 1979), and uptake of protozoa by other protozoa (Eq. 14.3 and 14.9) (review Coleman, 1986) was included. Analogously to the stabilizing effect of production of specific autoinhibitors on coexistence of populations (De Freitas & Fredrickson, 1978), this representation

Table 8. *Comparison of experimentally observed parameters (obs) and parameters predicted by the model (pred) for steers fed straw/tapioca based diets*

	Diet							
	U		GM		C		TC	
	obs	pred	obs	pred	obs	pred	obs	pred
Duodenal flow of NAN (g/d)	33.4	23.3	47.0	34.9	43.8	37.4	67.8	41.7
Rumen outflow of (% total NAN flow):								
Microbial NAN, RNA[1]	98		85		92		53	
Microbial NAN, AEPA+DAPA	144	87	102	71	108	72	73	54
Microbial NAN, mAA	89		75		84		54	
Protozoal NAN, RNA-DAPA	10		15		11		-4	
Protozoal NAN, AEPA	56	11	32	6	27	6	16	6
Protozoal NAN, mAA	25		14		9		25	
Bacterial NAN, DAPA	88	76	70	65	81	66	57	48
Rumen pool size of (g):								
Protozoal N	-	6.7	-	5.5	-	5.6	-	6.0
Microbial N	-	20.5	-	25.8	-	24.3	-	20.6

Observations from Cockburn & Williams (1984); Diet U, urea supplementation; Diet GM, groundnut meal supplementation; Diet C, untreated casein supplementation; Diet TC, formaldehyde treated casein; NAN, non-ammonia nitrogen.

[1] microbial markers used: AEPA, aminoethylphosphonic acid; DAPA, diaminopimelic acid; mAA, modified amino acid profile; RNA, ribonucleic acid.

of protozoal metabolism and interactions with bacteria allowed stable coexistence under a wide range of dietary inputs. It should be noted that there are at least two situations where simulated coexistence does not occur. Firstly, at high intake levels of concentrate diets, the high availability of easily fermentable substrates resulted in high protozoal death rates and in low pH values of rumen fluid and hence, both protozoa and cellulolytic bacteria disappeared. The results reported by Eadie *et al.* (1970) and Russell & Dombrowski (1980) support such a disappearance of protozoa and cellulolytic bacteria, respectively. Secondly, on diets without soluble sugars and all starch available in the insoluble form, amylolytic bacteria would be excluded (results not shown); however, such diets are unlikely to be fed (Tamminga *et al.* 1990).

Sensitivity analysis

Recycling of bacterial biomass is mainly due to protozoal predation (Wallace & McPherson, 1987), and the choice of values for the affinity constant for bacterial engulfment ($M_{Bx,BxPo}$; Figure 2A) and the inhibition constant with respect to protozoal storage polysaccharides ($J_{Sp,BxPo}$) had large effects on predicted bacterial biomass and degradation of protein and carbohydrates in the rumen. In earlier versions of the model, the inhibitory effect of protozoal storage polysaccharides on bacterial uptake (Coleman, 1975) was not represented and this resulted in too low simulated values of bacterial biomass in the rumen and degradation of OM. However, in the current model, these parameter values were assigned arbitrarily and further experiments to estimate these values are needed.

The model was very sensitive to variations in the maintenance requirement of protozoa ($R_{Xx,XxPm}$) and such variations gave rise to simulated disappearance of either bacteria or protozoa (Figure 2B). To the author's knowledge, there are no published data on protozoal maintenance requirements in literature. This value has been set at a value slightly higher than the maintenance requirement estimated for mixed bacteria *in vitro* reported by Isaacson *et al.* (1975). Some justification for this value can be obtained from the figures on rate of endogenous amylopectin utilization by holotrich protozoa reported by Prins & Van Hoven (1977) and Van Hoven & Prins (1977). On the assumption that the maximum amount of storage polysaccharides equals 40% of total protozoal DM (Czerkawski, 1976) and that the endogenous fermentation represents the maintenance requirement of microbes, values between 6.5×10^{-3} and 9.5×10^{-3} mol hexose/(g Po·d) can be calculated. The affinity constant related to death of protozoa ($M_{Va,PoDe}$) influenced microbial populations significantly. Again, this value was assigned arbitrarily. In the model, death rate is assumed to be related to rate of fermentation of hexoses within protozoa to represent the observed lysis of protozoa at high availability of easily fermentable substrates (Prins & Van Hoven, 1977; Williams, 1979). Such a representation ignores other reasons for protozoal lysis, including presence of lytic agents and of toxic chemicals and the effects of oxygen intake in feed (Leng, 1989). However, the behaviour of the model

at the diets used in sensitivity analysis is satisfactorily, showing large increases in amylolytic bacterial biomass and decreases in protozoal biomass when the availability of easily degradable carbohydrates was raised to a high level, which qualitatively compare with observed protozoal and bacterial numbers as well (Mackie *et al.* 1978). These sensitivity analyses highlighted the need for quantitative data on bacterial engulfment rate, protozoal maintenance requirement and death rate as affected by the diet.

Comparison between simulated and experimental values

Several methods have been proposed to estimate protozoal biomass in the rumen and protozoal outflow to the duodenum. Bauchop & Clarke (1976) and Amos & Akin (1978) reported close association of rumen entodiniomorphid protozoa to plant fragments and Abe *et al.* (1981) observed a large mass of holotrich protozoa attached to the reticulo-rumen wall. Thus, although there is a considerable volume of data on effects of dietary factors upon protozoal numbers present in the rumen, the number of protozoa in rumen fluid was suggested to be an unreliable indicator of protozoal biomass in the rumen (Leng, 1989). The use of AEPA as a protozoal marker has been questioned, as it is not only present in protozoa but in feed and bacteria as well (Ling & Buttery, 1978; Whitelaw *et al.* 1984). Protozoal outflow to the duodenum has been estimated from the difference between microbial NAN flow measured with a general microbial marker (e.g. ^{15}N, ^{35}S, RNA) and a bacterial marker (DAPA), but the value of this method to estimate protozoal outflow is uncertain due to large variation in estimated flows related to the markers applied (Oldham & Tamminga, 1980). Ling (1990) showed that intra-ruminal digestion of bacterial cell walls will overestimate bacterial outflow to the duodenum measured by DAPA, and hence, protozoal outflow as measured by difference between markers could be estimated to be negative (e.g. Cockburn & Williams, 1984). Isotope dilution techniques using ^{14}C-choline have been applied to measure protozoal dynamics in the rumen and its application has been discussed elsewhere (Czerkawski, 1987; Leng, 1989). On most diets, protozoal biomass in the rumen was adequately predicted when compared with values obtained by the ^{14}C-choline method (Tables 6 and 7). Unfortunately, all but one diets were based on oaten chaff and this limits the scope of comparison. Thus, further evaluation requires reliable estimates of protozoal biomass on a range of dietary conditions.

Simulated protozoal turnover time was not predicted well without any consistent pattern of over- or underestimation. Estimates of protozoal biomass and turnover time obtained with the ^{14}C-choline method are not independent, however. It was striking that, on most diets, an overestimated rumen protozoal pool size was accompanied by an overestimated turnover time and the reversed. Hence, the differences between simulated and observed protozoal production rates in the rumen (calculated as the reciprocal of turnover time multiplied by protozoal biomass and expressed in g protozoal N/d) were smaller than the differences between observed and simulated

turnover times. Simulated turnover times were calculated ignoring the turnover due to uptake of protozoa by other ones. Thus, 'true' turnover time would be lower than values stated in Tables 6 and 7. The concept that the majority of protozoa die and lyse in the rumen, as observed using the ^{14}C-choline method and as simulated, has been questioned by Czerkawski (1987), suggesting that a larger part of protozoal biomass flows out the rumen but is lysed in the omasum. Evidence reported by Michalowski & Harmeyer (1983) and Leng (1989) does not support this suggestion. The simulated proportion of protozoal NAN in microbial NAN outflow on the range of diets in Tables 5-7 was low (6-13%). Using direct counts, Weller & Pilgrim (1974) calculated that protozoal N outflow amounted 1-2% of dietary N intake only, and with the same method, Collombier *et al.* (1984) and Punia *et al.* (1987) calculated a contribution of 5 and 24-27% of protozoal to total microbial NAN flow, respectively. Steinhour *et al.* (1982), using a ^{15}N rate of incorporation method, calculated 33-51% of microbial NAN flow to be of protozoal origin. John & Ulyatt (1984) obtained much lower values (9-19%) using phosphatidyl choline to estimate protozoal flow. As discussed before, the method using the difference between a general microbial and a bacterial marker yields a wide range of estimates of protozoal NAN flows. Thus, most methods indicate a relatively low protozoal outflow, but for further evaluation, there is a real need for a valid protozoal marker.

Responses to changes in input parameters

The response of protozoal OM in the rumen to changes in fractional passage rate of fluids (k_{FlEx}) differed from that to changes in solid passage rate (k_{SoEx}) (Figure 3). Protozoal biomass was highest at intermediate values of k_{FlEx}, but decreased continuously with increased k_{SoEx}. Protozoal numbers or biomass in the rumen or *in vitro* have been reported to be moderately increased (Michalowski & Harmeyer, 1983), not changed (Crawford *et al.* 1980) or decreased (Leng *et al.* 1984) with increases in k_{FlEx}, and such dynamic behaviour is in line with the curvilinear responses predicted by the model. However, protozoal numbers or biomass are much more influenced by changes in k_{SoEx} than in k_{FlEx} and at high values of k_{SoEx}, protozoa have been observed to disappear (Abe & Kumeno, 1973; Crawford *et al.* 1980). Thus, model simulations stress the importance of the dynamic behaviour of protozoa within the rumen ecosystem with respect to passage rates.

The model provides the opportunity to change dietary components independently and study the effects on microbial populations in the rumen. In line with results reported in the review of Jouany (1989), simulated protozoal biomass was greatly influenced by variations in dietary starch, but was less affected by variations in dietary contents of sugars and did not change in response to variations in dietary NDF contents (Figure 4). However, responses to increases in dietary starch contents depended on the type of diet and the level of starch content. Increasing the starch content from 300 to 400 g/kg DM decreased protozoal biomass on diet C but increased protozoal biomass on diet R. The reason for the decreased protozoal

biomass on Diet C is a much higher increase in simulated protozoal death rate. Thus, the model provides a mechanism for examining the relationship between protozoal OM in the rumen and dietary composition, taking into account the dynamic behaviour of microbial populations in the rumen. Equally, supplementation of roughage diets with small amounts of soluble sugars increased protozoal biomass, while on concentrate diets protozoal biomass was decreased due to protozoal lysis. Simulations indicated that this difference between diets would have been even more pronounced, had the roughage diets contained no starch (results not shown). While bacterial biomass was increased with an improved ruminal N availability, protozoal biomass was hardly changed. This is the result of the assumption made in the model that, in most situations, and even when dietary protein content is low, protozoa do not lack N since sufficient protein is available from engulfed microbial matter. These simulations show the importance of including dynamic interactions between protozoa, bacteria and dietary characteristics in order to examine the effects of changes in dietary composition on microbiological parameters and degradation of OM in the rumen.

In simulations, changes in feed intake level had to be accompanied by changes in passage rates, rumen volume and pH of rumen fluid to prevent unrealistic model values of microbial biomass in the rumen, and such modifications have been reported in literature as well (Owens & Goetsch, 1986; Robinson *et al.* 1985 and 1987). *Ad lib* intake of high concentrate diets leads to low protozoal numbers in rumen fluid or even complete disappearance (Eadie *et al.* 1970; Slyter *et al.* 1970; Vance *et al.* 1972), but doubling the feed intake of a hay based diet increased protozoal numbers (Jouany, 1989). Thus, model simulations, which indicated that the effect of feed intake level on changes of the concentration of protozoal biomass in the rumen depended on dietary composition and basal feed intake level, are qualitatively in line with observations. The simulated death rates of protozoa were increased to a much larger extent by increases in feed intake with Diet C than with Diet R. With Diet C, maximal protozoal concentrations were reached at a lower level of intake. Thus, the model provides a mechanistic understanding of the changes in protozoal concentration resulting from changes in feed intake level and provide a quantitative description of such changes.

It should be noted that with increased intake levels, the proportion of amylolytic bacterial N to total bacterial N is increased. Because of higher fractional outflow of amylolytic than cellulolytic bacteria, these changes implicate that variations in the protozoal contribution to rumen microbial biomass are not necessarily accompanied by changes of the same relative size in protozoal contribution to microbial NAN flow. For example, on diet C, the contribution of protozoal N to rumen microbial N on the lowest and highest intake level differed only slightly (47 and 46% respectively), yet protozoal contribution to microbial NAN outflow varied more (18 and 12% respectively). Thus, estimations of proportional contributions of protozoa to microbial NAN flow varies with different levels of intake of a diet and cannot be based on

protozoal biomass in the rumen alone. Specific nutritional aspects of protozoa, including dissimilarities in amino acid profile of protozoal and bacterial protein (Buttery, 1977), selective retention within the rumen (review Jouany *et al.* 1988), and the general absence of propionic acid in protozoal fermentation endproducts (review Williams & Coleman, 1988), will cause differences in the profile of nutrients available for absorption. It is hoped that results of the model, giving a quantification of protozoal contribution to rumen microbial metabolic activities related to dietary characteristics, will encourage research into integrating protozoal activities to the profile of nutrients available for absorption.

In conclusion, the model provides a mathematical representation of the substrates and microbial populations in the rumen with special emphasis on protozoa. It gives a quantitative understanding of the protozoal dynamics and interactions with rumen bacteria as affected by dietary characteristics. Both very low and high availability of energy substrates resulted, by different mechanisms, in low protozoal levels in the rumen. The responses to changes in dietary composition and feed intake level have been given and differences in response to one particular aspect of dietary input have been discussed with respect to basal level of the other dietary inputs. The need for reliable estimates of protozoal biomass, turnover and outflow on a range of diets has been indicated, as well as the lack of quantitative data on bacterial engulfment and protozoal maintenance requirement and death rate.

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MATHEMATICAL APPENDIX

Table A1. *Symbols for entities and processes used in the model*

Symbol	Entity	Symbol	Entity
Ab	Absorption from rumen	Pd	Rumen degradable protein
Ac	Acetic acid	Pm	Protozoal maintenance
Am	Ammonia	Po	Protozoa
Ba	Amylolytic bacteria	Pr	Propionic acid
Bc	Cellulolytic bacteria	Ps	Rumen fluid soluble protein
Bx	Bacteria, general	Pu	Rumen undegradable protein
Bu	Butyric acid	Px	Engulfed protein
Ce	Cellulose	Ru	Rumen
De	Death of protozoa	Sa	Amylolytic bacterial storage polysaccharides
Ex	Exit from rumen into lower tract		
Fd	Rumen degradable neutral detergent fibre	Sd	Rumen degradable starch
Fe	Feed	Sf	Proportion of engulfed starch fermented for energy
Fl	Rumen fluids		
Fu	Rumen undegradable neutral detergent fibre	Sl	Saliva
Ha	Amylolytic hexose	So	Rumen solids
Hc	Cellulolytic hexose	Sp	Protozoal storage polysaccharides
He	Hemicellulose	Sr	Rumen fluid soluble starch
Hf	Proportion of hexose fermented for energy	St	Starch
In	Intake	Ue	Urea
La	Lactic acid	Va	Volatile fatty acids
Lc	Lactic acid carbohydrate equivalents	Vl	Valeric acid
Le	Lactic acid energy equivalents	Wr	Water soluble carbohydrates
Li	Lipids	Xf	Proportion of general engulfed hexose fermented for energy
Lx	Engulfed lipids		
Ni	Nitrogen	Xx	General engulfed hexose entity

Table A2. *General notation used in model*

Notation	Translation	Units
C_i	Concentration of *i*	(mol or g *i*)/l
D_i	Driving variable with respect to *i*	(g or l *i*)/d
$f_{i,j}$	Fraction of *i* in *j*	g *i*/kg *j* or -
$f_{i,jk}$	Fraction of *i* in *j-k* transaction	-
$J_{i,jk}$	Inhibition constant for *j-k* transaction with respect to *i*	mol *i*/l or -
ki,j	Mass action rate constant for *i-j* transaction	/d
$M_{i,jk}, M^*_{i,jk}$	Michaelis-Menten constants for *j-k* transaction with respect to *i*	(mol or g *i*)/l or -
$P_{i,jk}$	Rate of production of *i* by *j-k* transaction	(mol or g *i*)/d
$P_{mi,jk}$	Rate of production of *i* by *j-k* transaction due to *m* microbes	g *i*/d
Q_i	Quantity of *i*	mol or g *i*
$R_{i,jk}$	Requirement for *i* in *j-k* transaction	mol *i*/(mol or g *j*)
$\Phi_{i,jk}$	Steepness parameter associated with *i* for *j-k* transaction	-
t	Time	d
T_i	Digestion turnover time of feed component *i*	d
T^*_i	Reference digestion turnover time of feed component *i*	d
$U_{i,jk}$	Rate of utilization of *i* by *j-k* transaction	(mol or g *i*)/d
$U_{mi,jk}$	Rate of utilization of *i* by *j-k* transaction due to *m* microbes	mol *i*/d
V_i	Volume of *i*	l
v^*_{jk}	Maximum velocity for *j-k* transaction	(mol or g *j*)/(g or l)·d
v_{jk}	Velocity for *j-k* transaction	(mol or g *j*)/d
$v_{i,jk}$	Velocity for *j-k* transaction with respect to *i*	mol *j*/d
$Y_{i,jk}$	Yield of *i* for *j-k* transaction	(mol or g *i*)/(mol, g or l *j*)

i, *j*, *k* and *m* take values from Table A1.

Table A3. *Principal transactions occurring within the rumen*

Transaction	Substrate: principal; auxiliary	Products: principal; auxiliary
AmBa	Am; Ha	Ba; Ac, Pr, Bu, Vl
AmBc	Am; Hc	Bc; Ac, Pr, Bu, Vl
BaPo	Ba; Sp	Px; Po, Am, Ps, Ac, Pr, Bu, Vl
BcPo	Bc; Sp	Px; Po, Am, Ps, Ac, Pr, Bu, Vl
FdHc	Fd	Hc
FdPo	Fd; Sp	Po; Ac, Pr, Bu, Vl
HaSa	Ha; Sp	Sa; Ac, Pr, Bu, Vl
HaSp	Ha; Sp	Sp; Ac, Pr, Bu, Vl
HaVa	Ha	Ac, Pr, Bu, Vl
HcVa	Hc	Ac, Pr, Bu, Vl
PdPs	Pd	Ps
PdPo	Pd; Sp	Px; Am, Ps, Ac, Pr, Bu, Vl
PoDe	Po; Ac, Pr, Bu, Vl	Ps, Ha, Li
PoPo	Po; Sp	Px; Po, Am, Ps, Ac, Pr, Bu, Vl
PsAm	Ps; Ha, Hc	Am; Ac, Pr, Bu, Vl
PsBa	Ps; Ha	Ba; Ac, Pr, Bu, Vl
PsBc	Ps; Hc	Bc; Ac, Pr, Bu, Vl
PsPo	Ps	Px; Am, Ps, Ac, Pr, Bu, Vl
SaPo	Sa	Po; Ac, Pr, Bu, Vl
SdHa	Sd	Ha
SdPo	Sd	Po; Ac, Pr, Bu, Vl
SpDe	Sp	Ha
UeAm	Ue; Am	Am

See Tables A1 and A2 for explanation of notation.

Table A4. *Mathematical statement of model*

Protein and non-protein N digestion

Degradable protein pool, Q_{Pd} (mol)

Concentration:	$C_{Pd} = Q_{Pd}/V_{Ru}$	(1.1)
Inputs:	$P_{Pd,InPd} = Y_{Pd,InPd}D_{Pd}$	(1.2)
Outputs:	$U_{Pd,PdPs} = v_{PdPs}/[1 + M_{Pd,PdPs}/C_{Pd}]$	(1.3)
	$U_{Pd,PdPo} = v_{PdPo}/[1 + M_{Pd,PdPo}/C_{Pd}]$	(1.4)
	$U_{Pd,PdEx} = k_{PdEx}Q_{Pd}$	(1.5)
Differential eq:	$dQ_{Pd}/dt = P_{Pd,InPd} - U_{Pd,PdPs} - U_{Pd,PdPo} - U_{Pd,PdEx}$	(1.6)
Auxiliary eqns:	$v_{PdPs} = v^{*}_{PdPs}(Q_{Ba} + Q_{Bc})$	(1.7)
	$M_{Pd,PdPs} = M^{*}_{Pd,PdPs}T_{Pd}/T^{*}_{Pd}$	(1.8)

(continued)

Table A4 *(continued). Mathematical statement of model*

$$v_{PdPo} = v^{*}_{PdPo} Q_{Po} / [1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,PdPo})^{\Theta Sp,PdPo}] \quad (1.9)$$

$$M_{Pd,PdPo} = M^{*}_{Pd,PdPo} T_{Pd} / T^{*}_{Pd} \quad (1.10)$$

$$k_{PdEx} = k_{SoEx} \quad (1.11)$$

Soluble protein pool, Q_{Ps} (mol)

Concentration: $C_{Ps} = Q_{Ps}/V_{Ru}$ (2.1)

Inputs:
$$P_{Ps,InPs} = Y_{Ps,InPs} D_{Ps} \quad (2.2)$$
$$P_{Ps,SlPs} = Y_{Ps,SlPs} D_{Sl} \quad (2.3)$$
$$P_{Ps,PdPs} = Y_{Ps,PdPs} U_{Pd,PdPs} \quad (2.4)$$
$$P_{Ps,PxPs} = P_{Px,PxPo} - U_{Px,PxAm} - R_{Px,XxPo} P_{Po,XxPo} \quad (2.5)$$
$$P_{Ps,PoDe} = Y_{Ps,PoDe} U_{Po,PoDe} \quad (2.6)$$

Outputs:
$$U_{BaPs,PsAm} = v_{Ba,PsAm}/[1 + M_{Ps,PsAm}/C_{Ps} + C_{Ha}/J_{Ha,PsAm}] \quad (2.7)$$
$$U_{BcPs,PsAm} = v_{Bc,PsAm}/[1 + M_{Ps,PsAm}/C_{Ps} + C_{Hc}/J_{Hc,PsAm}] \quad (2.8)$$
$$U_{Ps,PsBa} = v_{PsBa}/[1 + M_{Ps,PsBa}/C_{Ps} + M_{Ha,PsBa}/C_{Ha}] \quad (2.9)$$
$$U_{Ps,PsBc} = v_{PsBc}/[1 + M_{Ps,PsBc}/C_{Ps} + M_{Hc,PsBc}/C_{Hc}] \quad (2.10)$$
$$U_{Ps,PsPo} = v_{PsPo}/[1 + M_{Ps,PsPo}/C_{Ps}] \quad (2.11)$$
$$U_{Ps,PsEx} = k_{PsEx} Q_{Ps} \quad (2.12)$$

Differential eq:
$$dQ_{Ps}/dt = P_{Ps,InPs} + P_{Ps,SlPs} + P_{Ps,PdPs} + P_{Ps,PxPs} + P_{Ps,PoDe} - U_{BaPs,PsAm} - U_{BcPs,PsAm} - U_{Ps,PsBa} - U_{Ps,PsBc} - U_{Ps,PsPo} - U_{Ps,PsEx} \quad (2.13)$$

Auxiliary eqns:
$$P_{Px,PxPo} = Y_{Px,BaPo} U_{Ba,BaPo} + Y_{Px,BcPo} U_{Bc,BcPo} + Y_{Px,PoPo} U_{Po,PoPo} + U_{Pd,PdPo} + U_{Ps,PsPo} \quad (2.14)$$
$$v_{Ba,PsAm} = v^{*}_{PsAm} Q_{Ba} \quad (2.15)$$
$$v_{Bc,PsAm} = v^{*}_{PsAm} Q_{Bc} \quad (2.16)$$
$$v_{PsBa} = v^{*}_{PsBa} Q_{Ba} \quad (2.17)$$
$$v_{PsBc} = v^{*}_{PsBc} Q_{Bc} \quad (2.18)$$
$$v_{PsPo} = v^{*}_{PsPo} Q_{Po} \quad (2.19)$$
$$k_{PsEx} = k_{FlEx} \quad (2.20)$$

Undegradable protein pool, Q_{Pu} (mol)

Concentration: $C_{Pu} = Q_{Pu}/V_{Ru}$ (3.1)

Input:
$$P_{Pu,InPu} = Y_{Pu,InPu} D_{Pu} \quad (3.2)$$

Output:
$$U_{Pu,PuEx} = k_{PuEx} Q_{Pu} \quad (3.3)$$

Differential eq:
$$dQ_{Pu}/dt = P_{Pu,InPu} - U_{Pu,PuEx} \quad (3.4)$$

Auxiliary eq:
$$k_{PuEx} = k_{SoEx} \quad (3.5)$$

Ammonia pool, Q_{Am} (mol)

Concentration: $C_{Am} = Q_{Am}/V_{Ru}$ (4.1)

Inputs:
$$P_{Am,InAm} = Y_{Am,InAm} D_{Am} \quad (4.2)$$
$$P_{Am,UeAm} = Y_{Am,UeAm} v_{Ue,Am} \quad (4.3)$$

(continued)

Table A4 *(continued). Mathematical statement of model*

$$P_{BaAm,PsAm} = Y_{Am,PsAm}U_{BaPs,PsAm} \quad (4.4)$$
$$P_{BcAm,PsAm} = Y_{Am,PsAm}U_{BcPs,PsAm} \quad (4.5)$$
$$P_{Am,PxAm} = Y_{Am,PxAm}U_{Px,PxAm} \quad (4.6)$$

Outputs:
$$U_{Am,AmBa} = v_{AmBa}/[1 + M_{Am,AmBa}/C_{Am} + M_{Ha,AmBa}/C_{Ha}] \quad (4.7)$$
$$U_{Am,AmBc} = v_{AmBc}/[1 + M_{Am,AmBc}/C_{Am} + M_{Hc,AmBc}/C_{Hc}] \quad (4.8)$$
$$U_{Am,AmAb} = v_{AmAb}/[1 + M_{Am,AmAb}/C_{Am}] \quad (4.9)$$
$$U_{Am,AmEx} = k_{AmEx}Q_{Am} \quad (4.10)$$

Differential eq:
$$dQ_{Am}/dt = P_{Am,InAm} + P_{Am,UeAm} + P_{BaAm,PsAm} + P_{BcAm,PsAm} + P_{Am,PxAm} - U_{Am,AmBa} - U_{Am,AmBc} - U_{Am,AmAb} - U_{Am,AmEx} \quad (4.11)$$

Auxiliary eqns:
$$v_{UeAm} = v^{*}_{UeAm}V_{Ru}f_{Ni,Fe}/(1 + C_{Am}/J_{Am,UeAm}) \quad (4.12)$$
$$U_{Px,PxAm} = v_{PxAm}Q_{Po}/[1 + M_{Px,PxAm}/(P_{Px,PxPo} - R_{Px,XxPo}P_{Po,XxPo})/Q_{Po}] \quad (4.13)$$
$$M_{Px,PxAm} = M^{*}_{Px,PxAm}/T^{*}_{Px} \quad (4.14)$$
$$v_{AmBa} = v^{*}_{AmBa}Q_{Ba} \quad (4.15)$$
$$v_{AmBc} = v^{*}_{AmBc}Q_{Bc} \quad (4.16)$$
$$v_{AmAb} = v^{*}_{AmAb}V_{Ru}^{0.75}/[1 + (M_{pH,AmAb}/pH)^{\Theta pH,AmAb}] \quad (4.17)$$
$$k_{AmEx} = k_{FlEx} \quad (4.18)$$

Lipid Digestion

Lipid pool, Q_{Li} (mol)

Concentration:
$$C_{Li} = Q_{Li}/V_{Ru} \quad (5.1)$$

Inputs:
$$P_{Li,InLi} = Y_{Li,InLi}D_{Li} \quad (5.2)$$
$$P_{Li,PoDe} = Y_{Li,PoDe}U_{Po,PoDe} \quad (5.3)$$
$$P_{Li,LxLi} = P_{Lx,LxPo} - R_{Lx,XxPo}P_{Po,XxPo} \quad (5.4)$$

Output:
$$U_{Li,LiPo} = v_{LiPo}/[1 + M_{Li,LiPo}/C_{Li}] \quad (5.5)$$
$$U_{Li,LiEx} = k_{LiEx}Q_{Li} \quad (5.6)$$

Differential eq:
$$dQ_{Li}/dt = P_{Li,InLi} + P_{Li,PoDe} + P_{Li,LxLi} - U_{Li,LiPo} - U_{Li,LiEx} \quad (5.7)$$

Auxiliary eq:
$$P_{Lx,LxPo} = Y_{Lx,BaPo}U_{Ba,BaPo} + Y_{Lx,BcPo}U_{Bc,BcPo} + Y_{Lx,PoPo}U_{Po,PoPo} + U_{Li,LiPo} \quad (5.8)$$
$$v_{LiPo} = v^{*}_{LiPo}Q_{Po} \quad (5.9)$$
$$k_{LiEx} = k_{SoEx} \quad (5.10)$$

Carbohydrate digestion

Degradable starch pool, Q_{Sd} (mol)

Concentration:
$$C_{Sd} = Q_{Sd}/V_{Ru} \quad (6.1)$$

Input:
$$P_{Sd,InSd} = Y_{Sd,InSd}D_{Sd} \quad (6.2)$$

(continued)

Table A4 *(continued). Mathematical statement of model*

Outputs:	$U_{Sd,SdHa} = v_{SdHa}/[1 + M_{Sd,SdHa}/C_{Sd}]$	(6.3)
	$U_{Sd,SdPo} = v_{SdPo}/[1 + M_{Sd,SdPo}/C_{Sd}]$	(6.4)
	$U_{Sd,SdSp} = v_{SdSp}/[1 + M_{Sd,SdSp}/C_{Sd}]$	(6.5)
	$U_{Sd,SdEx} = k_{SdEx}Q_{Sd}$	(6.6)
Differential eq:	$dQ_{Sd}/dt = P_{Sd,InSd} - U_{Sd,SdHa} - U_{Sd,SdPo} - U_{Sd,SdSp} - U_{Sd,SdEx}$	(6.7)
Auxiliary eqns:	$v_{SdHa} = v^{*}_{SdHa}Q_{Ba}$	(6.8)
	$M_{Sd,SdHa} = M^{*}_{Sd,SdHa}T_{Sd}/T^{*}_{Sd}$	(6.9)
	$v_{SdPo} = v^{*}_{SdPo}Q_{Po}$	(6.10)
	$M_{Sd,SdPo} = M^{*}_{Sd,SdPo}T_{Sd}/T^{*}_{Sd}$	(6.11)
	$v_{SdSp} = v^{*}_{SdSp}Q_{Po}/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,SdSp})^{\Theta Sp,SdSp}$	(6.12)
	$M_{Sd,SdSp} = M^{*}_{Sd,SdSp}T_{Sd}/T^{*}_{Sd}$	(6.13)
	$k_{SdEx} = k_{SoEx}$	(6.14)

Degradable fibre pool, Q_{Fd} (mol)

Concentration:	$C_{Fd} = Q_{Fd}/V_{Ru}$	(7.1)
Input:	$P_{Fd,InFd} = Y_{Fd,InFd}D_{Fd}$	(7.2)
Outputs:	$U_{Fd,FdHc} = v_{FdHc}/[1 + M_{Fd,FdHc}/C_{Fd}]$	(7.3)
	$U_{Fd,FdPo} = v_{FdPo}/[1 + M_{Fd,FdPo}/C_{Po}]$	(7.4)
	$U_{Fd,FdEx} = k_{FdEx}Q_{Fd}$	(7.5)
Differential eq:	$dQ_{Fd}/dt = P_{Fd,InFd} - U_{Fd,FdHc} - U_{Fd,FdPo} - U_{Fd,FdEx}$	(7.6)
Auxiliary eqns:	$v_{FdHc} = v^{*}_{FdHc}Q_{Bc}[(1 - tf/24) + (tf/24)$ $/[1 + (M_{pH,FdHc}/pm)^{\Theta pH,FdHc}]]$	(7.7)
	$M_{Fd,FdHc} = M^{*}_{Fd,FdHc}T_{Fd}/T^{*}_{Fd}$	(7.8)
	$v_{FdPo} = v^{*}_{FdPo}Q_{Po}/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,FdPo})^{\Theta Sp,FdPo}$	(7.9)
	$M_{Fd,FdPo} = M^{*}_{Fd,FdPo}T_{Fd}/T^{*}_{Fd}$	(7.10)
	$k_{FdEx} = k_{SoEx}$	(7.11)

Undegradable fibre pool, Q_{Fu} (mol)

Concentration:	$C_{Fu} = Q_{Fu}/V_{Ru}$	(8.1)
Input:	$P_{Fu,InFu} = Y_{Fu,InFu}D_{Fu}$	(8.2)
Output:	$U_{Fu,FuEx} = k_{FuEx}Q_{Fu}$	(8.3)
Differential eq:	$dQ_{Fu}/dt = P_{Fu,InFu} - U_{Fu,FuEx}$	(8.4)
Auxiliary eq:	$k_{FuEx} = k_{SoEx}$	(8.5)

Amylolytic hexose pool, Q_{Ha} (mol)

Concentration:	$C_{Ha} = Q_{Ha}/V_{Ru}$	(9.1)
Inputs:	$P_{Ha,WrHa} = Y_{Ha,WrHa}D_{Wr}$	(9.2)
	$P_{Ha,SrHa} = Y_{Ha,SrHa}D_{Sr}$	(9.3)
	$P_{Ha,LaHa} = Y_{Ha,LaHa}D_{La}$	(9.4)
	$P_{Ha,InLi} = Y_{Ha,InLi}D_{Li}$	(9.5)

(continued)

Table A4 *(continued). Mathematical statement of model*

$$P_{Ha,SdHa} = Y_{Ha,SdHa}U_{Sd,SdHa} \quad (9.6)$$

$$P_{Ha,PoDe} = Y_{Ha,PoDe}U_{Po,PoDe} \quad (9.7)$$

$$P_{Ha,SpDe} = Y_{Ha,SpDe}U_{Sp,SpDe} \quad (9.8)$$

Outputs:

$$U_{Ha,AmBa} = R_{Ha,AmBa}U_{Am,AmBa} \quad (9.9)$$

$$U_{Ha,PsBa} = R_{Ha,PsBa}U_{Ps,PsBa} \quad (9.10)$$

$$U_{Ha,HaSa} = v_{HaSa}/[1 + M_{Ha,HaSa}/C_{Ha}] \quad (9.11)$$

$$U_{Ha,HaVa} = v_{HaVa}/[(1 + M_{Ha,HaVa}/C_{Ha})(1 + C_{Am}/J_{Am,HaVa} + C_{Ps}/J_{Ps,HaVa})] \quad (9.12)$$

$$U_{Ha,HaPo} = v_{HaPo}/[1 + M_{Ha,HaPo}/C_{Ha}] - U_{Ha,HaSp} \quad (9.13)$$

$$U_{Ha,HaSp} = v_{HaSp}/[1 + M_{Ha,HaSp}/C_{Ha}] \quad (9.14)$$

$$U_{Ha,HaEx} = k_{HaEx}Q_{Ha} \quad (9.15)$$

Differential eq:

$$dQ_{Ha}/dt = P_{Ha,WrHa} + P_{Ha,SrHa} + P_{Ha,LaHa} + P_{Ha,InLi} + P_{Ha,SdHa} + P_{Ha,PoDe} + P_{Ha,SpDe} - U_{Ha,AmBa} - U_{Ha,PsBa} - U_{Ha,HaSa} - U_{Ha,HaVa} - U_{Ha,HaPo} - U_{Ha,HaSp} - U_{Ha,HaEx} \quad (9.16)$$

Auxiliary eqns:

$$v_{HaSa} = v^{*}_{HaSa}Q_{Ba} \quad (9.17)$$

$$v_{HaVa} = v^{*}_{HaVa}Q_{Ba} \quad (9.18)$$

$$v_{HaPo} = v^{*}_{HaPo}Q_{Po} \quad (9.19)$$

$$v_{HaSp} = v^{*}_{HaSp}Q_{Po}/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,HaSp})^{\Theta Sp,HaSp}] \quad (9.20)$$

$$k_{HaEx} = k_{FlEx} \quad (9.21)$$

Cellulolytic hexose pool, Q_{Hc} (mol)

Concentration:

$$C_{Hc} = Q_{Hc}/V_{Ru} \quad (10.1)$$

Inputs:

$$P_{Hc,FdHc} = Y_{Hc,FdHc}U_{Fd,FdHc} \quad (10.2)$$

Outputs:

$$U_{Hc,AmBc} = R_{Hc,AmBc}U_{Am,AmBc} \quad (10.3)$$

$$U_{Hc,PsBc} = R_{Hc,PsBc}U_{Ps,PsBc} \quad (10.4)$$

$$U_{Hc,HcVa} = v_{HcVa}/[(1 + M_{Hc,HcVa}/C_{Hc})(1 + C_{Am}/J_{Am,HcVa} + C_{Ps}/J_{Ps,HcVa})] \quad (10.5)$$

$$U_{Hc,HcEx} = k_{HcEx}Q_{Hc} \quad (10.6)$$

Differential eq:

$$dQ_{Ha}/dt = P_{Hc,FdHc} - U_{Hc,AmBc} - U_{Hc,PsBc} - U_{Hc,HcVa} - U_{Hc,HcEx} \quad (10.7)$$

Auxiliary eqns:

$$v_{HcVa} = v^{*}_{HcVa}Q_{Bc} \quad (10.8)$$

$$k_{HcEx} = k_{FlEx} \quad (10.9)$$

Microbial growth, passage and death

Amylolytic bacteria pool, Q_{Ba} (g)

Concentration:

$$C_{Ba} = Q_{Ba}/V_{Ru} \quad (11.1)$$

Inputs:

$$P_{Ba,AmBa} = Y_{Ba,AmBa}U_{Am,AmBa} \quad (11.2)$$

$$P_{Ba,PsBa} = Y_{Ba,PsBa}U_{Ps,PsBa} \quad (11.3)$$

Outputs:

$$U_{Ba,BaPo} = v_{BaPo}/[1 + M_{Bx,BxPo}/(C_{Ba} + C_{Bc} + C_{Sa})] \quad (11.4)$$

(continued)

Table A4 *(continued). Mathematical statement of model*

$$U_{Ba,BaEx} = k_{BaEx}Q_{Ba} \quad (11.5)$$

Differential eq: $dQ_{Ba}/dt = P_{Ba,AmBa} + P_{Ba,PsBa} - U_{Ba,BaPo} - U_{Ba,BaEx}$ (11.6)

Auxiliary eqns: $v_{BaPo} = v^{*}_{BxPo}Q_{Po}C_{Ba}/[C_{Ba} + C_{Bc} + C_{Sa}]/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,BxPo})^{\Theta Sp,BxPo}]$ (11.7)

$$k_{BaEx} = k_{FlEx} \quad (11.8)$$

Amylolytic bacterial storage polysaccharides pool, Q_{Sa} (g)

Concentration: $C_{Sa} = Q_{Sa}/V_{Ru}$ (12.1)

Input: $P_{Sa,HaSa} = Y_{Sa,HaSa}U_{Ha,HaSa}$ (12.2)

Outputs: $U_{Sa,SaPo} = v_{SaPo}/[1 + M_{Bx,BxPo}/(C_{Ba} + C_{Bc} + C_{Sa})]$ (12.3)

$$U_{Sa,SaEx} = k_{SaEx}Q_{Sa} \quad (12.4)$$

Differential eq: $dQ_{Sa}/dt = P_{Sa,HaSa} - U_{Sa,SaPo} - U_{Sa,SaEx}$ (12.5)

Auxiliary eq: $v_{SaPo} = v^{*}_{BxPo}Q_{Po}C_{Sa}/[C_{Ba} + C_{Bc} + C_{Sa}]/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,BxPo})^{\Theta Sp,BxPo}]$ (12.6)

$$k_{SaEx} = k_{FlEx} \quad (12.7)$$

Cellulolytic bacteria pool, Q_{Bc} (g)

Concentration: $C_{Bc} = Q_{Bc}/V_{Ru}$ (13.1)

Inputs: $P_{Bc,AmBc} = Y_{Bc,AmBc}U_{Am,AmBc}$ (13.2)

$$P_{Bc,PsBc} = Y_{Bc,PsBc}U_{Ps,PsBc} \quad (13.3)$$

Outputs: $U_{Bc,BcPo} = v_{BcPo}/[1 + M_{Bx,BxPo}/(C_{Ba} + C_{Bc} + C_{Sa})]$ (13.4)

$$U_{Bc,BcEx} = k_{BcEx}Q_{Bc} \quad (13.5)$$

Differential eq: $dQ_{Bc}/dt = P_{Bc,AmBc} + P_{Bc,PsBc} - U_{Bc,BcPo} - U_{Bc,BcEx}$ (13.6)

Auxiliary eqns: $v_{BcPo} = v^{*}_{BxPo}Q_{Po}C_{Bc}/[C_{Ba} + C_{Bc} + C_{Sa}]/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,BxPo})^{\Theta Sp,BxPo}]$ (13.7)

$$k_{BcEx} = k_{SoEx} \quad (13.8)$$

Protozoa pool, Q_{Po} (g)

Concentration: $C_{Po} = Q_{Po}/V_{Ru}$ (14.1)

Inputs: $P_{Po,XxPo} = MIN[(Y_{Po,XxPo}(U_{Xx,XxPo} - U_{Xx,XxPm})), (P_{Px,PxPo}/R_{Px,XxPo})]$ (14.2)

Outputs: $U_{Po,PoPo} = v_{PoPo}/[1 + M_{Po,PoPo}/(C_{Po} + C_{Sp})]$ (14.3)

$$U_{Po,PoDe} = v_{PoDe} \quad (14.4)$$

$$U_{Po,PoEx} = k_{PoEx}Q_{Po} \quad (14.5)$$

Differential eq: $dQ_{Po}/dt = P_{Po,XxPo} - U_{Po,PoPo} - U_{Po,PoDe} - U_{Po,PoEx}$ (14.6)

Auxiliary eqns: $U_{Xx,XxPo} = U_{Fd,FdPo} + U_{Sd,SdPo} + U_{Ha,HaPo} + Y_{Xx,SaPo}U_{Sa,SaPo} + Y_{Xx,SpPo}U_{Sp,SpPo} + Y_{Xx,BaPo}U_{Ba,BaPo} + Y_{Xx,BcPo}U_{Bc,BcPo} + Y_{Xx,PoPo}U_{Po,PoPo}$ (14.7)

$$U_{Xx,XxPm} = R_{Xx,XxPm}Q_{Po} \quad (14.8)$$

$$v_{PoPo} = v^{*}_{PoPo}Q_{Po}C_{Po}/(C_{Po} + C_{Sp})/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,PoPo})^{\Theta Sp,PoPo}] \quad (14.9)$$

(continued)

Table A4 *(continued). Mathematical statement of model*

$$v_{PoDe} = v^{*}_{PoDe}Q_{Po}/[1 + (M_{Va,PoDe}/P_{Va,XxVa}/Q_{Po})^{\Theta Va,PoDe}] \quad (14.10)$$

$$P_{Va,XxVa} = P_{Ac,SdSp} + P_{Ac,HaSp} + P_{Ac,XxPo} + P_{Pr,SdSp} + P_{Pr,HaSp} + P_{Pr,XxPo} + P_{Bu,SdSp} + P_{Bu,HaSp} + P_{Bu,XxPo} + P_{Vl,SdSp} + P_{Vl,HaSp} + P_{Vl,XxPo} \quad (14.11)$$

$$M_{Va,PoDe} = M^{*}_{Va,PoDe}/T_{Va} \quad (14.12)$$

$$k_{PoEx} = 0.45k_{SoEx} \quad (14.13)$$

Protozoal storage polysaccharides pool, Q_{Sp} (g)

Concentration: $C_{Sp} = Q_{Sp}/V_{Ru}$ (15.1)

Inputs: $P_{Sp,HaSp} = Y_{Sp,HaSp}U_{Ha,HaSp}$ (15.2)

$P_{Sp,SdSp} = Y_{Sp,SdSp}U_{Sd,SdSp}$ (15.3)

Outputs: $U_{Sp,SpPo} = v_{SpPo}/[1 + M_{Po,PoPo}/(C_{Sp} + C_{Po}]$ (15.4)

$U_{Sp,SpDe} = v_{SpDe}$ (15.5)

$U_{Sp,SpEx} = k_{SpEx}Q_{Sp}$ (15.6)

Differential eq: $dQ_{Sp}/dt = P_{Sp,HaSp} + P_{Sp,SdSp} - U_{Sp,SpPo} - U_{Sp,SpDe} - U_{Sp,SpEx}$ (15.7)

Auxiliary eqns: $v_{SpPo} = v^{*}_{PoPo}Q_{Sp}C_{Sp}/(C_{Sp} + C_{Po})/[1 + (Q_{Sp}/(Q_{Sp} + Q_{Po})/J_{Sp,PoPo})^{\Theta Sp,PoPo}$ (15.8)

$v_{SpDe} = v^{*}_{PoDe}Q_{Sp}/[1 + (M_{Va,PoDe}/P_{Va,XxVa}/Q_{Po})^{\Theta Va,PoDe}]$ (15.9)

$k_{SpEx} = 0.45k_{SoEx}$ (15.10)

Volatile fatty acids

Acetic acid pool, Q_{Ac} (mol)

Concentration: $C_{Ac} = Q_{Ac}/V_{Ru}$ (16.1)

Inputs: $P_{Ac,InAc} = Y_{Ac,InAc}D_{Ac}$ (16.2)

$P_{Ac,AmBa} = Y_{Ac,AmBa}U_{Hf,AmBa}$ (16.3)

$P_{Ac,AmBc} = Y_{Ac,AmBc}U_{Hf,AmBc}$ (16.4)

$P_{Ac,PsBa} = Y_{Ac,PsBa}U_{Hf,PsBa}$ (16.5)

$P_{Ac,PsBc} = Y_{Ac,PsBc}U_{Hf,PsBc}$ (16.6)

$P_{Ac,HaSa} = Y_{Ac,HaSa}U_{Hf,HaSa}$ (16.7)

$P_{Ac,HaVa} = Y_{Ac,HaVa}U_{Ha,HaVa}$ (16.8)

$P_{Ac,HcVa} = Y_{Ac,HcVa}U_{Hc,HcVa}$ (16.9)

$P_{BaAc,PsAm} = Y_{Ac,PsAm}U_{BaPs,PsAm}$ (16.10)

$P_{BcAc,PsAm} = Y_{Ac,PsAm}U_{BcPs,PsAm}$ (16.11)

$P_{Ac,PxAm} = Y_{Ac,PxAm}U_{Px,PxAm}$ (16.12)

$P_{Ac,HaSp} = Y_{Ac,HaSp}U_{Hf,HaSp}$ (16.13)

$P_{Ac,SdSp} = Y_{Ac,SdSp}U_{Sf,SdSp}$ (16.14)

$P_{Ac,XxPo} = Y_{Ac,XxPo}U_{Xf,XxPo}$ (16.15)

Outputs: $U_{Ac,AcAb} = v_{AcAb}/[1 + M_{Ac,AcAb}/C_{Ac}]$ (16.16)

$U_{Ac,AcEx} = k_{AcEx}Q_{Ac}$ (16.17)

(continued)

Table A4 *(continued). Mathematical statement of model*

Differential eq: $$dQ_{Ac}/dt = P_{Ac,InAc} + P_{Ac,AmBa} + P_{Ac,AmBc} + P_{Ac,PsBa} + P_{Ac,PsBc} + P_{Ac,HaSa} + P_{Ac,HaVa} + P_{Ac,HcVa} + P_{BaAc,PsAm} + P_{BcAc,PsAm} + P_{Ac,PxAm} + P_{Ac,HaSp} + P_{Ac,SdSp} + P_{Ac,XxPo} - U_{Ac,AcAb} - U_{Ac,AcEx} \quad (16.18)$$

Auxiliary eqns:
$$U_{Hf,AmBa} = f_{Hf,AmBa}U_{Ha,AmBa} \quad (16.19)$$
$$U_{Hf,AmBc} = f_{Hf,AmBc}U_{Hc,AmBc} \quad (16.20)$$
$$U_{Hf,PsBa} = f_{Hf,PsBa}U_{Ha,PsBa} \quad (16.21)$$
$$U_{Hf,PsBc} = f_{Hf,PsBc}U_{Hc,PsBc} \quad (16.22)$$
$$U_{Hf,HaSa} = f_{Hf,HaSa}U_{Ha,HaSa} \quad (16.23)$$
$$U_{Sf,SdSp} = f_{Sf,SdSp}U_{Sd,SdSp} \quad (16.24)$$
$$U_{Hf,HaSp} = f_{Hf,HaSp}U_{Ha,HaSp} \quad (16.25)$$
$$U_{Xf,XxPo} = U_{Xx,XxPm} + U_{Xx,XxVa} + f_{Xf,XxPo}(U_{Xx,XxPo} - U_{Xx,XxPm} - U_{Xx,XxVa}) \quad (16.26)$$
$$Y_{Ac,LaAc} = f_{Lc,Le}Y_{Ac,WrAc} \quad (16.27)$$
$$Y_{Ac,HaVa} = (Y_{Ac,WrAc}(P_{Ha,WrHa} + P_{Ha,InLi} + P_{Ha,PoDe}) + Y_{Ac,LaAc}P_{Ha,LaHa} + Y_{Ac,StAc}(P_{Ha,SrHa} + P_{Ha,SdHa} + P_{Ha,SpDe}))/(P_{Ha,WrHa} + P_{Ha,LaHa} + P_{Ha,InLi} + P_{Ha,SrHa} + P_{Ha,SdHa} + P_{Ha,PoDe} + P_{Ha,SpDe}) \quad (16.28)$$
$$Y_{Ac,HcVa} = f_{Ce,Fd}Y_{Ac,CeAc} + (1 - f_{Ce,Fd})Y_{Ac,HeAc} \quad (16.29)$$
$$Y_{Ac,PsVa} = Y_{Ac,PsAc} \quad (16.30)$$
$$Y_{Ac,AmBa} = Y_{Ac,HaVa} \quad (16.31)$$
$$Y_{Ac,AmBc} = Y_{Ac,HcVa} \quad (16.32)$$
$$Y_{Ac,PsBa} = Y_{Ac,HaVa} \quad (16.33)$$
$$Y_{Ac,PsBc} = Y_{Ac,HcVa} \quad (16.34)$$
$$Y_{Ac,HaSa} = Y_{Ac,HaVa} \quad (16.35)$$
$$Y_{Ac,PsAm} = Y_{Ac,PsVa} \quad (16.36)$$
$$Y_{Ac,PxAm} = Y_{Ac,PsVa} \quad (16.37)$$
$$Y_{Ac,SdSp} = Y_{Ac,StAc} \quad (16.38)$$
$$Y_{Ac,HaSp} = Y_{Ac,HaVa} \quad (16.39)$$
$$Y_{Ac,FdVa} = f_{Ce,Fd}Y_{Ac,CeAc} + (1 - f_{Ce,Fd})Y_{Ac,HeAc} \quad (16.40)$$
$$Y_{Ac,XxPo} = (Y_{Ac,FdVa}U_{Fd,FdPo} + Y_{Ac,StVa}(U_{Sd,SdPo} + Y_{Xx,SaPo}U_{Sa,SaPo} + Y_{Xx,SpPo}U_{Sp,SpPo}) + Y_{Ac,HaVa}U_{Ha,HaPo} + Y_{Ac,WrAc}(Y_{Xx,BaPo}U_{Ba,BaPo} + Y_{Xx,BcPo}U_{Bc,BcPo} + Y_{Xx,PoPo}U_{Po,PoPo}))/U_{Xx,XxPo} \quad (16.41)$$
$$U_{Xx,XxVa} = MAX[((U_{Xx,XxPo} - U_{Xx,XxPm})Y_{Po,XxPo} - (P_{Px,PxPo}/R_{Px,XxPo}))/Y_{PoXxPo}, 0.0] \quad (16.42)$$
$$v_{AcAb} = v^{*}_{AcAb}V_{Ru}^{0.75}/[1 + (pH/J_{pH,AcAb})^{\Theta pH,AcAb}] \quad (16.43)$$
$$k_{AcEx} = k_{FlEx} \quad (16.44)$$

(continued)

Table A4 *(continued). Mathematical statement of model*

Propionic acid pool, Q_{Pr} (mol)

Equations for this pool as for acetic acid pool but with Pr replacing Ac throughout

Butyric acid pool, Q_{Bu} (mol)

Equations for this pool as for acetic acid pool but with Bu replacing Ac throughout

Valeric acid pool, Q_{Vl} (mol)

Equations for this pool as for acetic acid pool but with Vl replacing Ac throughout

See Tables A1 and A2 for explanation of notation.

Chapter 5

SIMULATION OF THE EFFECTS OF DIET ON THE CONTRIBUTION OF RUMEN PROTOZOA TO DEGRADATION OF FIBRE IN THE RUMEN

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SIMULATION OF THE EFFECTS OF DIET ON THE CONTRIBUTION OF RUMEN PROTOZOA TO DEGRADATION OF FIBRE IN THE RUMEN

JAN DIJKSTRA AND SEERP TAMMINGA

A previously described mathematical model, that simulates the metabolic activities of rumen bacteria and protozoa, was used to examine the contribution of protozoa to NDF degradation in the rumen of cattle. Comparisons between predicted and experimentally observed NDF degradation and protozoal N in the rumen showed general agreement. Further simulations were performed with diets, containing variable proportions of concentrate (between 0 and 100% of diet DM) and at intake levels ranging between 5.3 and 21.0 kg DM/d. Except for the all concentrate diets, raising the feed intake level of these diets reduced the simulated protozoal contribution to NDF degradation from 17-21% with an intake of 5.3 kg DM/d to 5-13% with an intake of 21.0 kg DM/d. Predicted ruminal NDF degradation with the all concentrate diet at the highest intake level was only 8% and entirely related to protozoal activity. The changes in contribution of protozoa to NDF degradation were related to variations of the ratio of cellulolytic bacteria to protozoa and of the NDF degrading activities of protozoa predicted by the model. In simulations where dietary NDF levels were reduced and starch and sugar levels were increased independently, protozoal contribution to NDF degradation generally increased. These differences were also reflected in the increased protozoal contribution to NDF degradation generally predicted in response to a decreased roughage:concentrate ratio. The contribution of protozoa generally declined in response to added nitrogen as well. These changes in predicted protozoal contribution to NDF degradation resulting from dietary variations provided possible explanations for the differences in rumen NDF degradation observed when animals are defaunated.

Rumen: Computer simulation: Mathematical model: Ruminants: Fibre: Protozoa

Forages, which generally contain large amounts of cell wall carbohydrates, form the basis of a ruminant diet. The rumen is the major site of degradation of cell wall carbohydrates (Van Soest, 1982). A mixed population of rumen microorganisms, including fungi, protozoa and bacteria, are involved in fibre degradation. Fungi are a recently discovered group of microorganisms, contributing up to 8% of microbial biomass (Orpin, 1984; Fonty *et al.* 1988). Ciliate protozoa are generally present in large numbers, their biomass at times approximating or even exceeding that of bacteria (Hungate, 1966). Both protozoa and bacteria are of clear significance in rumen metabolism. In contrast with the considerable knowledge on predominant rumen cellulolytic bacteria, the role of rumen protozoa in fibre degradation is still

controversial (see reviews Coleman, 1986; Williams, 1986; Jouany, 1989). Study of the contribution of protozoa to fibre degradation is severely hampered by the inability to cultivate protozoa *in vitro* routinely in absence of growing bacteria. The study of defaunated animals could provide an alternative, but reported results are controversial (Ushida *et al.* 1991). The effect of defaunation of the rumen on rumen fibre degradation varies between over 50% inhibition to 15% stimulation (review Demeyer, 1989). Results of biochemical, cultural and microscopic studies indicate that the contribution of protozoa depends on the complex interactions between protozoa, bacteria and dietary characteristics (review Jouany, 1989). Thus, attempts to explain the non-systematic modifications resulting from removal of protozoa should include these relationships. An increased understanding of interactions between several components of a biological system needs an integration approach, which presently is only possible through mathematical representation of the processes involved (Thornley & Johnson, 1990).

The aim of the present study was to establish the likely roles of the rumen protozoa in fibre degradation on a wide range of dietary inputs, using a mathematical model of rumen fermentation (Dijkstra, submitted).

MATERIAL AND METHODS

Model description, general

A full description of the mathematical model, including the assumptions on protozoal and bacterial metabolism, the mathematical representation of microbial metabolism, and derivation of maximum reaction velocities, affinity and inhibition constants have been described previously (Dijkstra *et al.* 1992 and Dijkstra, submitted). The model is driven by continuous inputs of nutrients, calculated from the amount of feed fed and the chemical composition of the diet, including estimates of solubility, degradability and digestion turnover times of feed components, and by fractional outflow rates of fluid and solid phases as well as rumen fluid pH. The model comprises 19 state variables, each representing a pool size in the rumen. State variables relate to the carbohydrate entities (rumen degradable and undegradable fibre, starch and mono- or disaccharides derived from hydrolysis of fibre, starch and sugars), nitrogen containing entities (rumen degradable and undegradable protein and ammonia), fatty acid containing entities (lipid and volatile fatty acids) and microbial entities (amylolytic bacteria, cellulolytic bacteria and protozoa). The majority of the transaction kinetics were described using standard expressions from enzyme kinetics (Michaelis-Menten equations). Within the objective of the model, nutrients other than carbon or nitrogen containing substrates were assumed to be present in non-limiting amounts. The computer program was written in the simulation language ACSL (Mitchell & Gauthier, 1981) and the model was solved by integration of 19 state variables with a fourth-order Runge-Kutta method.

Representation of fibre degradation

A brief description of the representation of fibre utilization by rumen bacteria and protozoa is given; full details can be found elsewhere (Dijkstra *et al.* 1992; Dijkstra, submitted). In view of substrate preference shown by many bacterial species, within the model amylolytic bacteria and cellulolytic bacteria have been represented separately. The first group utilized hexose derived from starch and sugars, the latter utilized hexose derived from fibre. Protozoa utilized both fibre as well as starch and sugars, but maximum rate of fibre utilization was assumed to be lower than maximum rate of starch or soluble sugar utilization. The presence of storage polysaccharides within protozoa was assumed to inhibit uptake rate of insoluble material, including fibre. Fibre degradation by the cellulolytic bacteria was assumed to be inhibited at low pH values (pH < 6.3). Increased lysis of protozoa on diets rich in easily degradable carbohydrates, which are generally accompanied by low pH values of rumen fluid, has been included in the model.

Simulated diets

The simulations were conducted for dairy cows and were designed to study the effects of different intake levels, variations in proportions of concentrate in the diet and changes in dietary levels of Neutral Detergent Fibre (NDF), starch, sugars, and nitrogen (N) on protozoal contribution to degradation of NDF in the rumen. The input values were based on the study of Robinson *et al.* (1986 and 1987). In this experiment, dairy cows (with an average body weight of 583 kg) were fed diets of 1/3 ryegrass hay and 2/3 concentrate, containing different proportions of starch, at intake levels of 5.3, 9.2, 13.1, 17.1 and 21.0 kg DM/d. Simulations were performed for the intermediate level of starch in the concentrate, and the chemical composition of the hay and the concentrate is given in Table 1 (adopted from Robinson *et al.* 1986). Estimates of soluble and undegradable fractions and digestion turnover times were calculated as described before (Dijkstra *et al.* 1992). Fluid and solid passage rates, rumen volume, and rumen fluid pH at the different intake levels were adopted from Robinson *et al.* (1986 and 1987) as well.

The effect of the roughage:concentrate ratio at several intake levels was investigated using the same hay and concentrates (Table 1), with proportions of

Table 1. *Chemical composition (g/kg DM) of the hay and concentrate used in simulations*

	Hay	Concentrate
Neutral Detergent Fibre	520	355
Soluble sugars	160	68
Starch	0	197
Crude fat	23	31
Total nitrogen	21.7	28.9

Adopted from Robinson *et al.* (1986).

concentrate in the diet set at 0, 33, 67 and 100%. Changes in fluid and solid passage rates were estimated according to the relative changes calculated using the regression equations reported in the review of Owens and Goetsch (1986). For each intake level, pH values of the 0 and 33% concentrate diets were assumed to be 0.2 and 0.1 unit higher, and of the 100% concentrate diet 0.1 unit lower than the corresponding value of the 67% concentrate diet reported by Robinson *et al.* (1987). Some justification for these assumptions on pH changes are the values of mean and minimum pH reported by Bosch (1991) on silage based diets (mean and minimum pH decrease of 0.2 and 0.3 units respectively when dietary concentrate proportion increased from 6 to 36%, accompanied by an increase of DM intake from 13.3 to 17.3 kg/d).

To assess the effect of the individual feed components, the amount of NDF, starch, sugars, and N was varied while keeping all other values constant. The variation range chosen was: NDF, 300-600 g/kg DM; starch, 0-300 g/kg DM; sugars, 0-300 g/kg DM; N, 17.5-32.5 g/kg DM. Results are reported for four diets: a 0% concentrate diet at an intake level of 5.3 (Diet RL) or 17.1 (Diet RH) kg DM/d, and a 67% concentrate diet at an intake level of 5.3 (Diet RH) or 17.1 (Diet CH) kg DM/d.

The simulated contribution of rumen protozoa to fibre degradation was calculated as the amount of NDF taken up and subsequently degraded within the protozoa, divided by the total amount of NDF degraded in the rumen.

RESULTS

Comparison between predicted and observed values

In Table 2, observed parameters (reported by Robinson *et al.* 1986 and 1987) and parameters predicted by the model are presented for 5 intake levels, as well as the error of predicted relative to observed values (root Mean Square Prediction Error, MSPE; Bibby and Toutenburg, 1977). It should be noted that Robinson *et al.* (1987) calculated duodenal NDF flow by dividing faecal NDF flow by 0.85, based on studies of Tamminga (1981). Except for the 9.2 kg DM/d intake level, there was a close agreement for duodenal NDF flow between observed and simulated results, with a root MSPE of 3.5%. Both observed and simulated values indicated a decline in rumen NDF degradation as intake increased. As observed in the previous model (Neal *et al.* 1992), rumen ammonia concentration was seen to be overpredicted with a MSPE of 66%. Both observed and predicted values showed an increase of total VFA concentration of rumen fluid as intake increased (MSPE = 10.4%). Rumen microbial N was observed and predicted to increase with each feed intake increment. The generally overpredicted microbial N probably can be attributed to fact that Robinson *et al.* (1987) used DAPA to estimate microbial N in the rumen, which is assumed to measure bacterial biomass only, while predictions of microbial N include protozoal N as well. The lowest simulated proportion of cellulolytic bacterial OM and highest proportion of protozoal OM in the rumen occurred with an intake level of 9.2 kg

Table 2. *Comparison of experimentally observed parameters (obs) and parameters predicted by the model (pred), and error of predicted values relative to observed values (MSPE), for cows fed diets at different intake levels*

	Intake level (kg DM/d)										MSPE
	5.3		9.2		13.1		17.1		21.0		
	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred	
Duodenal flow of OM (g/d):											
NDF	0.79	0.81	1.45	1.63	2.18	2.17	3.42	3.44	4.24	4.23	3.5
Total NAN	-	125	-	226	-	323	-	437	-	521	-
Bacterial NAN	-	63	-	107	-	158	-	201	-	252	-
Protozoal NAN	-	6	-	11	-	13	-	16	-	16	-
Concentration of (mM):											
Ammonia	6.9	8.8	5.3	8.5	6.7	10.4	6.4	8.3	5.0	12.0	66.1
Total VFA	93	73	106	94	124	112	119	117	131	127	10.4
Microbial N in rumen (g)	87	91	128	121	157	169	168	173	183	226	10.4
Microbial OM (% of total):											
Cellulolytic bacterial	-	49.3	-	47.5	-	53.8	-	53.4	-	56.2	-
Protozoal	-	24.8	-	26.0	-	23.7	-	23.6	-	21.0	-
Protozoal NDF degradation (% of total degradation)	-	16.7	-	16.8	-	14.0	-	12.9	-	12.6	-

Observations from Robinson *et al.* (1986 and 1987); DM, dry matter; MSPE, mean square prediction error; NAN, non-ammonia nitrogen; NDF, neutral detergent fibre; OM, organic matter; VFA, volatile fatty acid.

DM/d, and at this level the simulated contribution of protozoa to NDF degradation was 16.8% and decreased to 12.6% at the highest intake level.

Effect of the intake level and roughage:concentrate ratio

The predicted amount of protozoal OM in the rumen, the proportion of rumen protozoal OM in the total amount of cellulolytic bacterial and protozoal OM, and the contribution of protozoa to fibre degradation in the rumen, with diets containing 0, 33, 67 or 100% of concentrate and fed at 5 intake levels, are shown in Figures 1A, 1B and 1C, respectively. Protozoal OM generally increased in response to increased feeding levels (Figure 1A). The predicted small elevation or even drop in protozoal OM when feed intake was raised from 13.1 to 17.1 kg DM/d is the result of the relatively high solid passage rate at the 17.1 kg DM/d level, adopted from Robinson *et al.* (1987). Protozoal OM was always lowest with the all roughage diet and generally increased when concentrate replaced roughage. However, with higher intake levels,

the increased availability of easily fermentable carbohydrates on the all concentrate diets caused fractional death rate of protozoa to increase rapidly (see equations 14.10 - 14.12 of the computer program; Dijkstra, submitted), the result being that predicted protozoal OM with the all concentrate diet was lower than the 67% concentrate diet for intake levels exceeding 12 kg DM/d. The proportion of protozoal OM in the total cellulolytic microbial OM (the latter consisting of cellulolytic bacteria and protozoa) was generally predicted to decline with an increase in feed intake, but with the all concentrate diet, this proportion was increased (Figure 1B). Intake of the all concentrate diet exceeding 15 kg DM/d resulted in predicted elimination of cellulolytic bacteria, due to the relatively low amount of substrate available and the low pH of rumen fluid. Hence, the proportion of predicted protozoal OM to cellulolytic microbial OM was 1. Thus, high intake of the all concentrate diet resulted in a low ruminal NDF degradation (only 8% of intake), entirely related to the metabolic activity of rumen protozoa (Figure 1C). With lower inclusions of concentrate in the diet, the highest protozoal contribution to NDF degradation at the low intake level was predicted to occur with the all roughage diet (21, 20, 17% with 0, 33 and 67% concentrate, respectively). By contrast, when feed intake level was increased up to 21 kg DM/d, this contribution with the all roughage diet declined to 5%, while on the 67% concentrate diet, the decline was much less pronounced (13% with highest feed intake level). The pattern of contribution of protozoa to rumen fibre degradation qualitatively matched the pattern of the proportion of protozoal OM in total cellulolytic microbial OM. However, except for the situation where cellulolytic bacteria are predicted to disappear, the NDF degradation activity of protozoa per unit protozoal OM was always lower than the corresponding activity of cellulolytic bacteria, which can be seen from the ratio between the contribution of protozoal OM to total cellulolytic microbial OM in the rumen and the contribution of protozoa to rumen NDF degradation. This relative NDF degradation activity of protozoa decreased in response to an increased dietary concentrate level as well, particularly with low levels of feed intake.

The effect of chemical composition of the feed

In this series of simulations, the effects of variations in chemical composition of the diet (NDF, starch, sugars, N) were investigated by varying the amount present without changing any other input parameter in the model. The predicted responses of protozoal OM in the rumen, the proportion of protozoal OM in the total cellulolytic microbial OM, and the contribution of protozoa to NDF degradation, to variations in NDF content of the diet are shown in Figure 2A, 2B and 2C, respectively, for all roughage (R) or 67% concentrate (C) diets fed at low (L; 5.3 kg DM/d) or high (H; 17.1 kg DM/d) intake level. Protozoal OM in the rumen was predicted to be unaffected by increases in the dietary NDF content (Figure 2A), whereas there was an increase in cellulolytic bacterial OM, because substrate availability was increased. Consequently, with all diets the proportion of protozoal OM in cellulolytic microbial

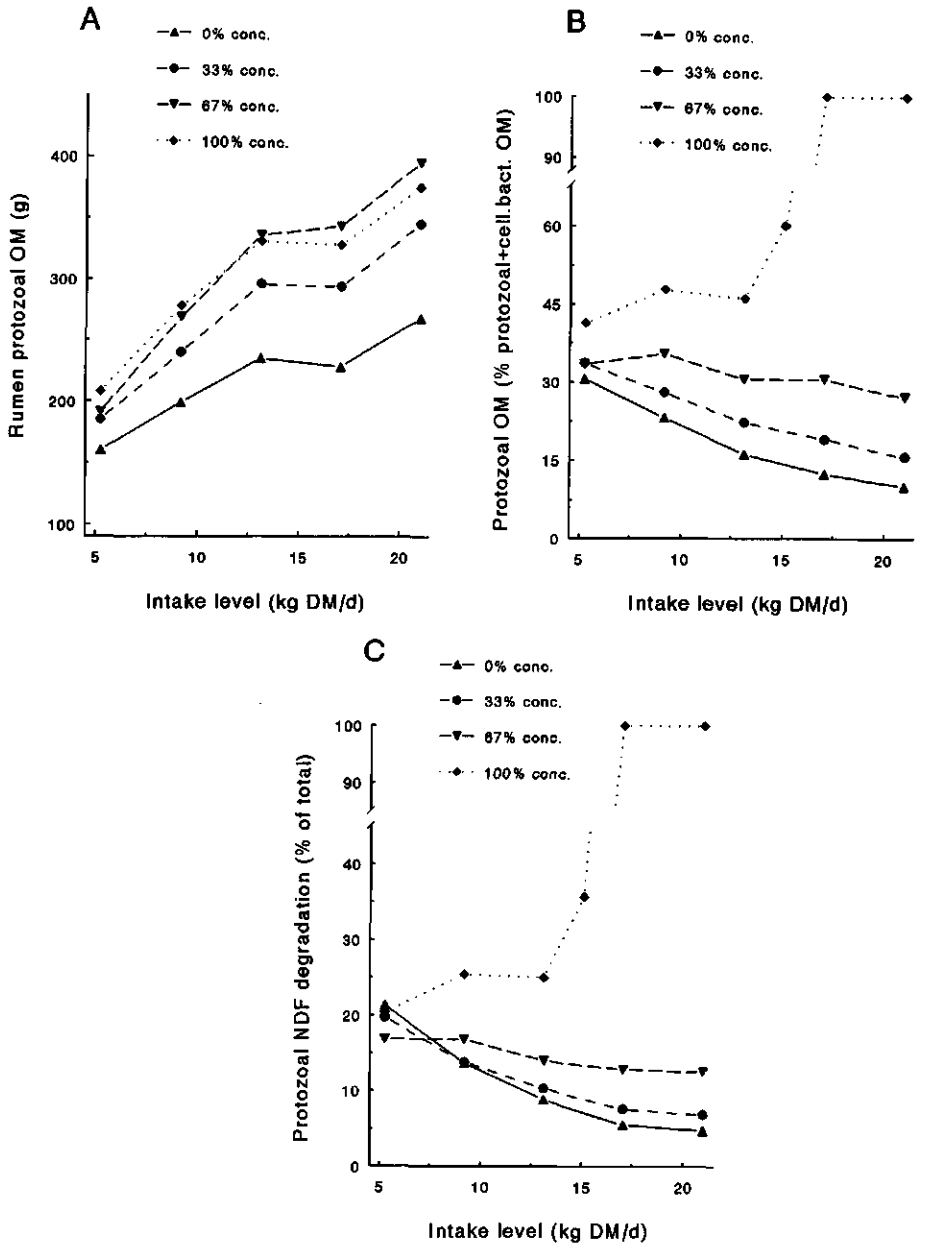

Figure 1. Predicted effect on protozoal OM in the rumen (A), protozoal OM in total cellulolytic microbial OM in the rumen (B) and protozoal contribution to NDF degradation in the rumen (C) of increasing DM intake level of diets containing 0, 33, 67 or 100% concentrate.

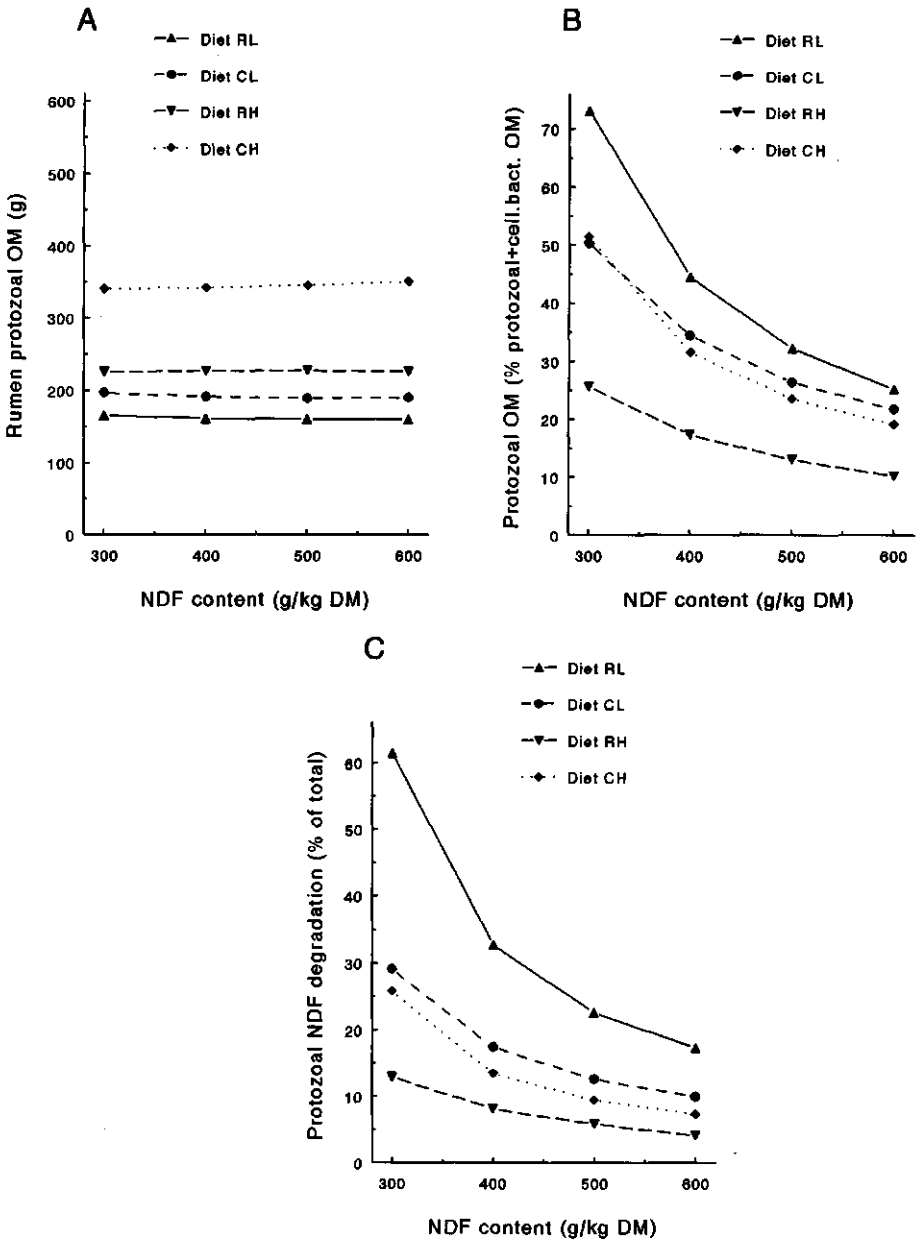

Figure 2. Predicted effect on protozoal OM in the rumen (A), protozoal OM in total cellulolytic microbial OM in the rumen (B) and protozoal contribution to NDF degradation in the rumen (C) of increasing dietary NDF level of all roughage diets fed at 5.3 kg DM/d (Diet RL) or 17.1 kg DM/d (Diet RH) and of 67% concentrate diets fed at 5.3 kg DM/d (Diet CL) or 17.1 kg DM/d (Diet CH).

OM decreased (Figure 2B), as well as the contribution of protozoa to fibre degradation (Figure 2C). These changes were most pronounced on the low intake, all roughage diet (Diet RL), and particularly so with low NDF levels. With Diet RL, the contribution of protozoa to fibre degradation decreased from 61% with 300 g NDF/kg DM to 33% with 400 g NDF/kg DM, and declined to 17% with 600 g NDF/kg DM.

In contrast with the predicted response of protozoal OM to variations in dietary NDF level, increases in dietary starch level resulted in elevated rumen protozoal OM. The relationship between starch content and protozoal OM in the rumen was almost linear, except for Diet CH (Figure 3A). With this particular diet, the relationship curved at the higher starch levels. This reduced response of protozoal OM resulted mainly from a rapid increase in fractional death rate, related to high availability of easily degradable substrates. Except for the initial decrease on Diet CL, the proportion of protozoal OM in cellulolytic microbial OM decreased (Figure 3B). The predicted contribution of protozoa to NDF degradation (Figure 3C) tended to a minimum value around a level of 100 g starch/kg DM. The model predicted that small additions of starch to a diet lacking starch increased the amount of storage polysaccharides within protozoa, and thus reduced the amount of particulate material engulfed, including fibre and cellulolytic bacteria (see equations 7.9 and 13.7 of the computer program; Dijkstra, submitted). This results in a reduction in protozoal contribution to fibre degradation. Further additions of starch did increase the protozoal contribution to NDF degradation, because the decline in protozoal contribution to NDF degradation due to increased protozoal storage material was more than compensated for by an increase in protozoal OM.

The effects of variations in dietary sugar content on protozoal OM depended on roughage:concentrate ratio as well as feeding level (Figure 4A). When the all roughage diet, devoid of starch, was fed and the soluble sugar content set at 0 g/kg DM, protozoa were predicted to disappear completely. The sole substrate available (NDF) did not support sufficient protozoal growth to prevent disappearance through death and passage to omasum. Hence, with Diets RL and RH, cellulolytic bacteria were the only cellulolytic microorganisms present (Figure 4B), and NDF degradation was related entirely to bacterial activity (Figure 4C). Incidently, due to the absence of preferred substrate, amylolytic bacteria were eliminated as well. Small additions of sugars rapidly increased predicted protozoal OM, and the contribution of protozoa to NDF degradation when sugar content was set at 100 g/kg DM was raised to 20 and 6% with diets RL and RH, respectively. Further sugar additions however, caused fractional death rate of protozoa to increase, and protozoal contribution to NDF degradation increased (Diet RL) or decreased (Diet RH) slightly. With Diet CH, the decline in protozoal OM was most pronounced (Figure 4A). Yet the protozoal contribution to NDF degradation was increased slightly (12 and 15% with 0 and 300 g sugar/kg DM respectively), because at high sugar availability, the partitioning of utilization of sugars for protozoal growth or protozoal storage material formation was shifted in favour of the former (see equations 9.13 and 9.14 of the computer

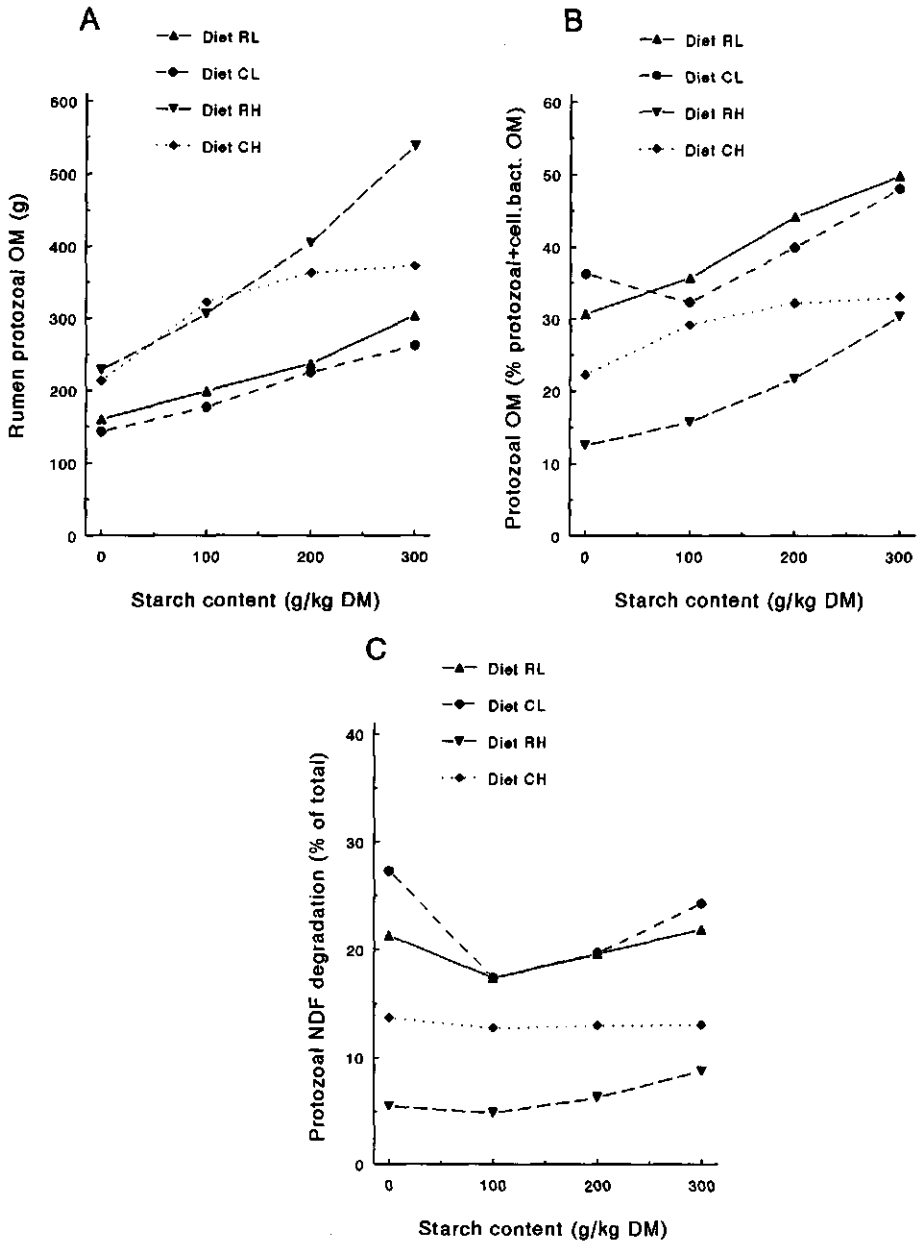

Figure 3. Predicted effect on protozoal OM in the rumen (A), protozoal OM in total cellulolytic microbial OM in the rumen (B) and protozoal contribution to NDF degradation in the rumen (C) of increasing dietary starch level of all roughage diets fed at 5.3 kg DM/d (Diet RL) or 17.1 kg DM/d (Diet RH) and of 67% concentrate diets fed at 5.3 kg DM/d (Diet CL) or 17.1 kg DM/d (Diet CH).

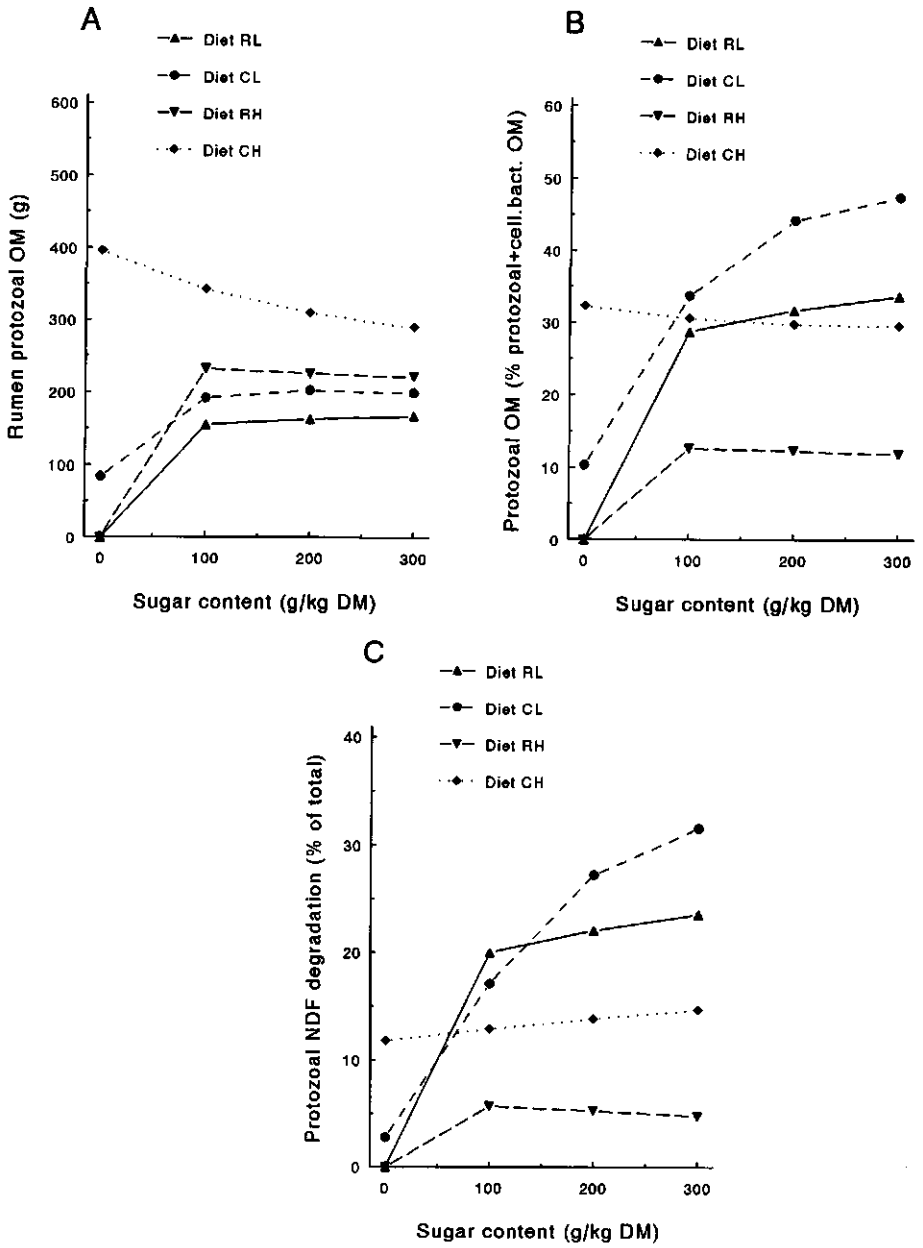

Figure 4. Predicted effect on protozoal OM in the rumen (A), protozoal OM in total cellulolytic microbial OM in the rumen (B) and protozoal contribution to NDF degradation in the rumen (C) of increasing dietary sugar level of all roughage diets fed at 5.3 kg DM/d (Diet RL) or 17.1 kg DM/d (Diet RH) and of 67% concentrate diets fed at 5.3 kg DM/d (Diet CL) or 17.1 kg DM/d (Diet CH).

program; Dijkstra, submitted). This resulted in a decreased amount of storage material within the protozoa and a small increase in protozoal NDF degradation. The effect was much more pronounced when this high concentrate diet was fed at a low level (Diet CL; protozoal contribution increased from 3 to 31% with dietary sugar contents of 0 and 300 g/kg DM), because protozoal OM did not decrease with increases in the dietary sugar level, as was the case with Diet CH.

Variations in the N content of the diet between 17.5 and 32.5 g/kg DM (i.e. crude protein (N x 6.25) levels between 11 and 20%) generally reduced the predicted amount of protozoal OM slightly (Figure 5A), whereas cellulolytic bacterial OM increased. Hence, a decreased proportion of protozoal OM in cellulolytic microbial OM was predicted in response to increases in dietary N level (Figure 5B). These changes in amounts of protozoal and bacterial OM are related to the assumptions in the model with respect to N utilization by protozoa and bacteria. Protozoa were assumed to engulf both bacterial and feed protein, and the supply of amino acids was generally sufficient, even so that a part of the engulfed protein was released into rumen fluid (Dijkstra, submitted). Besides, it was assumed that fermentation of protein does not yield a significant amount of ATP to be utilized for growth. By contrast, particularly with low N levels, bacteria would grow more efficient in response to raised N levels. This is because the amount of energy not related to growth decreased (see equations 9.12 and 10.5 of the computer program; Dijkstra, submitted), and the utilization of preformed amino acids for growth relative to utilization of ammonia increased slightly, which in turn reduced the amount of hexose needed per unit microbial growth. Thus, except for Diet RH, increments in dietary N level decreased the contribution of protozoa to NDF degradation (from 22, 23 and 15% with 17.5 g N/kg DM to 20, 11 and 12% with 32.5 g N/kg DM and Diets RL, CL and CH, respectively; Figure 5C). The small rise in protozoal contribution with Diet RH (5 and 6% with the lowest and highest N level, respectively) can be explained by the involvement of amylolytic bacteria. At the lowest N level, the predicted low availability of ammonia and amino acids in rumen fluid reduced amylolytic bacterial growth to a large extent. This resulted in a high substrate availability (particularly sugars) for protozoal growth and in turn in a very high protozoal death rate, reducing protozoal OM as well. Increasing the N level increased bacterial growth and reduced substrate supply to protozoa and consequently protozoal growth. However, this reduction was more than compensated for by the reduction in protozoal fractional death rate, which finally resulted in increased protozoal OM. This increased the amount of cellulolytic bacteria engulfed. Hence, although cellulolytic bacterial growth with Diet RH was more efficient with higher dietary N levels, the amount of cellulolytic bacterial OM declined.

DISCUSSION

To our knowledge, there are no published *in vivo* data on the amounts of NDF degraded in the rumen by protozoa, relative to the amounts degraded by bacteria,

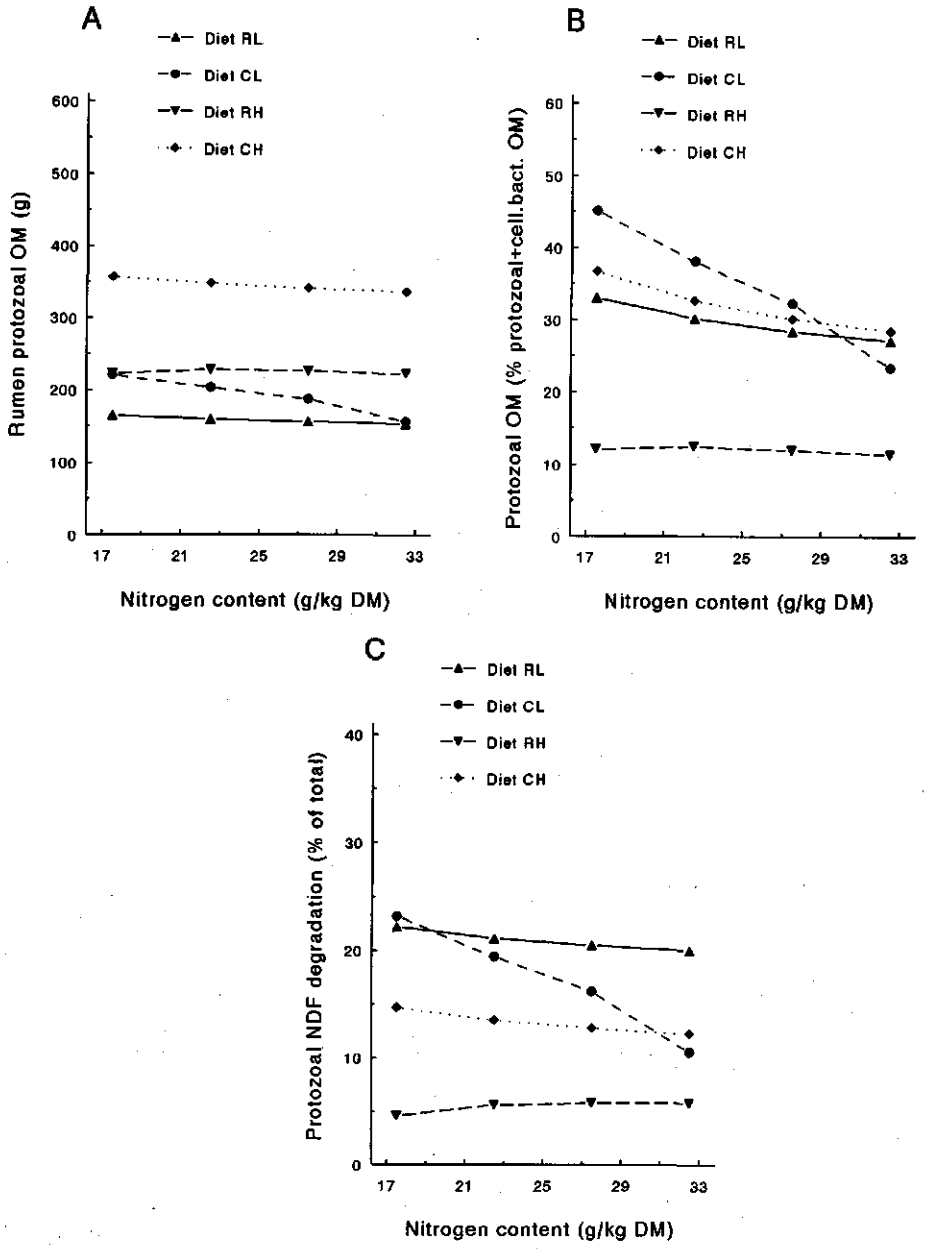

Figure 5. Predicted effect on protozoal OM in the rumen (A), protozoal OM in total cellulolytic microbial OM in the rumen (B) and protozoal contribution to NDF degradation in the rumen (C) of increasing dietary nitrogen level of all roughage diets fed at 5.3 kg DM/d (Diet RL) or 17.1 kg DM/d (Diet RH) and of 67% concentrate diets fed at 5.3 kg DM/d (Diet CL) or 17.1 kg DM/d (Diet CH).

when both protozoa and bacteria are present in the rumen. Estimates of *in vitro* DM disappearance of forages related to protozoal activity have been reported (Amos & Akin, 1978), as well as protozoal enzymatic activity against cell wall components (reviews Williams, 1986; Jouany, 1989). In some studies, the effect of defaunation on NDF or ADF degradation in the rumen have been reported (reviews Demeyer, 1989; Jouany, 1989; Ushida *et al.* 1991). These results will be discussed later in relation to the results of the simulations reported in the present paper. In the following sections the assumptions in the model with respect to NDF degradation and simulated patterns of protozoal and bacterial OM in response to dietary changes will be discussed.

Validation of a previous model, on which the present model was largely based, against a wide range of dietary inputs, showed that the degradation of NDF in the rumen was predicted satisfactorily (Neal *et al.* 1992). Equally, the results presented in Table 2 showed that predicted NDF degradation matched corresponding values estimated from NDF faecal flow, reported by Robinson *et al.* (1987) with DM intake levels ranging between 5.3 and 21.0 kg/d. In the model, it was assumed that both protozoa and bacteria are involved in the degradation of NDF. This is a simplification of the rumen ecosystem, because fungi might have a significant role in degradation of fibre in the rumen (review Fonty *et al.* 1988). Thus, the non-protozoal contribution to fibre degradation consisted of the contribution of both cellulolytic bacteria and of fungi. However, quantitative data on growth and metabolism of rumen fungi are very scarce and hence the metabolic activity of this group was not included in the model. Besides, validation of the model was with experimental results with a high quality diet and it is assumed that under such dietary conditions fungi do not play an important role in the rumen ecosystem (Grenet *et al.* 1989). The NDF degrading activities of rumen bacteria and protozoa were assumed to be directly proportional to (amongst others) the amounts of polysaccharide-free microbial matter present. Although such a representation is common to models of rumen fermentation, it should be noted that metabolic activity of microorganisms varies within and between species, and amounts of microorganisms alone are not necessarily the most vital measure to evaluate the metabolic significance of a group of organisms within an ecosystem (Brock, 1966). Differences in cell wall degrading activities between cellulolytic species (eg the lower cellulolytic activity of *Ruminococcus flavefaciens* compared with *R. albus*; Van Gylswyck & Labuschagne, 1971) and protozoal species (eg general absence or low activity of cellulases and hemicellulases in *Entodinium* species and holotrich protozoa and higher activities in larger Entodiniomorphids; Jouany, 1989) have largely not been accounted for in view of absence of quantitative data on individual species and problems accounted previously in representing several species within an ecosystem. Reichl & Baldwin (1976) defined eight microbial groups in their linear programming model of the rumen, but from considerable simplifications of the predicted rumen ecosystem these authors concluded that additional data and concepts with regard to microbial metabolism and microbial interactions were required. In the present model,

several assumptions with regard to microbial metabolic parameters had to be made due to lack of adequate data. From these considerations, it seemed inappropriate to distinguish more than three microbial groups, as has been represented in the present model. Some differences in NDF degrading activity of rumen protozoa have been represented, however. It was assumed that uptake and subsequent degradation of NDF by protozoa was inhibited in presence of storage polysaccharide material within the protozoa (equation 7.9 of the computer program; Dijkstra, submitted). Thus, the predicted rate of NDF degradation per gram protozoal OM per day decreased with higher amounts of storage polysaccharides within the protozoa, possibly reflecting a shift in protozoal species with species differences in cellulase and hemicellulase activity. In simulations where dietary starch content was varied (Figure 3), protozoal NDF degradation at zero starch levels was 14.0, 14.6, 9.4 and 13.9 mmol per gram protozoal OM per day with Diets RL, CL, RH and CH, respectively, whereas increases of dietary starch content to 300 g/kg DM decreased these values to 6.6, 7.3, 6.0 and 7.6 mmol per gram protozoal OM per day. This decline in NDF degradation activity might reflect the relative large increase of numbers of *Entodinium* spp., generally observed when diets rich in starch are fed compared with all roughage diets (Jouany, 1989), and the low cellulolytic and hemicellulolytic activity of these species. Small additions of soluble sugars decreased protozoal NDF degrading activity as well. However, particularly with high concentrate diets, further increases in dietary soluble sugar content slightly increased protozoal NDF degrading activity. Such an initial decrease in NDF degrading activity might be related to the rapid proportional increase of holotrich protozoa generally observed when soluble sugars are added to a diet, and the relative inactivity of holotrichs against cellulose and hemicellulose (Jouany, 1989). Diets rich in sugars caused lysis of holotrich protozoa, related to the inability to regulate the uptake rate of soluble sugars (review Williams, 1986) and consequently, the remaining protozoal population might have a higher NDF degrading activity.

Comparisons between model predictions and experimental observations, using the ^{14}C dilution technique in cattle and sheep, indicated reasonable agreement for protozoal biomass in the rumen (Dijkstra, submitted). Increasing the concentrate content of the diet increased the protozoal OM in the rumen at all intake levels, except for the all concentrate diet fed at higher intake levels (Figure 1A), and resulted in a higher protozoal proportion in total cellulolytic microbial OM in the rumen (Figure 1B). Predicted responses of protozoal and cellulolytic microorganisms to changes in chemical composition of the diet (Figures 2-5) indicated that the effects of roughage:concentrate ratio were mainly related to the NDF and starch content of the diet. The predictions were qualitatively in line with observations on dietary effects on numbers of protozoa in the rumen. Large protozoal populations are generally found with diets consisting of equal amounts of roughages and concentrates, whereas high feeding levels of high grain diets reduced protozoal numbers or even eliminated protozoa (review Jouany, 1989). Recently however, Towne *et al.* (1990) reported

that protozoa constituted a significant fraction of total rumen microbial population of beef cattle fed high grain diets *ad lib*, resulting in low pH values of rumen fluid. Besides, the predicted disappearance of cellulolytic bacteria with all concentrate diets at higher intake levels (Figure 1B), on which pH of rumen fluid is expected to be low, is supported by the complete washout of cellulolytic bacterial species observed *in vitro* at low pH values (Russell & Dombrowski, 1980). It should be noted though, that the concentrate on which the simulations were based contained a relatively large amount of fibrous by-products, and was characterized by a high NDF content (35.5%) and a relatively low starch and sugars content (27.1%) (Robinson *et al.* 1986). The predicted effects of the roughage:concentrate ratio would be more pronounced, had the starch and sugar level of the concentrate been higher, as can be seen from the predicted increase in protozoal OM when dietary starch (Figure 2A) and, to a lower extent, dietary sugar content (Figure 3A) was increased. The ability of rumen protozoa to utilize both dietary and microbial proteins had important consequences for distribution of microbial biomass in the rumen in response to changes in dietary N levels (Figure 4A and B). Predicted protozoal OM was often slightly reduced and cellulolytic bacterial OM raised when dietary N level was increased. Indeed, *in vivo*, protozoal N in the rumen was lower on a diet supplemented with casein (1.4 g protozoal N; Leng, 1982) compared with the unsupplemented diet (2.4 g protozoal N; Leng *et al.* 1984). Decreasing the N availability of soya bean meal by treatment with formaldehyde slightly reduced (though not significantly) protozoal N pool size and increased fluid-phase bacterial N pool size (Krebs *et al.* 1989). Overall, these simulations in which dietary components and composition were varied, showed qualitative agreement with observations.

The previously discussed simulated variations in NDF degrading activity of rumen protozoa and variations in ratios of protozoa to cellulolytic bacteria caused marked differences in the contribution of protozoa to NDF degradation. Save the all concentrate diet, higher feed intake levels resulted in lower contributions of rumen protozoa to NDF degradation (Figure 1C). At the lowest intake level, this contribution varied between 17 and 21%. Increasing the intake level to 21 kg DM/d reduced the protozoal contribution to NDF degradation to 5-13%. With the all concentrate diet, cellulolytic bacteria were predicted to disappear and at the highest intake level, ruminal NDF degradation was only 8%. Such low NDF degradation values with all concentrate diets or high intake levels have been reported in literature. Chamberlain & Thomas (1979) fed an all barley/maize diet to sheep and recorded 13% of cellulose intake to be degraded in the rumen. Ruminal degradation of ADF with a 26% haylage, 19% corn silage, 53% concentrate (mainly barley) diet, fed to dairy cattle at an intake level of 21 kg DM/d was 11-14% (McCarthy *et al.* 1989). The model predicted an increased contribution of protozoa to NDF degradation in response to replacement of roughages by concentrates, except with low intake levels. However, simulations in which dietary chemical composition was varied, indicated that protozoal contribution at the low feed intake level might have been raised with an increase in

concentrate level, had the concentrate contained less NDF and more starch (confirmed by simulations of which results are not shown). The contribution of protozoa to fibre degradation generally decreased in response to added N as well (Figure 5C).

It should be noted that model predictions of NDF degradation merely represented NDF engulfed by and degraded within protozoal cells, without indication of the origin (bacterial or protozoal) of enzymes involved. Williams & Coleman (1988) stated in their review, that some authors have found indirect evidence that at least a proportion of these enzymes is of protozoal origin, but also stated that other authors have questioned these findings. As discussed previously, differences between protozoal species in cellulase activity have been reported. More than 70% or less than 35% of carboxylmethylcellulase activity was associated with the protozoa in sheep containing either *Eudiplodinium maggii* or *Entodinium caudatum*, respectively (see review Williams & Coleman, 1988). *In vitro*, the degradation of cellulose was improved when protozoa were added to a suspension of bacteria. Additions of autoclaved or frozen and thawed protozoa however, increased cellulose degradation as well, though to a smaller extent (Yoder *et al.* 1966), suggesting that factors or mechanisms other than the relevant protozoal enzymes were involved too. Thus, both the direct protozoal hemicellulolytic and cellulolytic enzyme activities, as well as other protozoal characteristics, including engulfment of bacteria, competition for substrates and stabilizing effects on rumen fluid characteristics, are of importance in the relative contributions of protozoa and bacteria to degradation of cell wall material. The integrative nature of these processes and the inability to determine protozoal contribution directly in the faunated rumen have led to different opinions on the importance of protozoa in fibre degradation. Hungate (1975) stated that the amount of cellulose digested by rumen protozoa is small compared to that digested by bacteria, whereas Coleman (1988) reported the opposite. The results of the simulations indicate a contribution of 5-35% in cattle fed a variety of diets at intake levels below 15 kg DM/d. With more extreme diets, protozoal contributions of 0% (when diets are devoid of starch and soluble sugars) or 100% (high concentrate diets at high intake levels) have been simulated. Comparatively, fractionating studies in continuous artificial systems revealed that ciliates accounted for 19-28% of total cellulase activity on a mixture of filter paper cellulose and ground alfalfa (Gijzen *et al.* 1988). Yoder *et al.* (1966) reported a cellulose digestion coefficient of 7% with washed suspensions of protozoa *in vitro*, and Amos & Akin (1978) reported that rumen protozoa degraded 4-11% of intact orchardgrass and bermudagrass tissue.

Demeyer (1981) calculated from data on the effects of defaunation on plant cell wall degradation *in vitro* or *in sacco* that protozoa were responsible for 30-40% of total fibre degradation, a value which has been quoted frequently by other authors (eg Jouany *et al.* 1988; Williams & Coleman, 1988). However, this is not necessarily the direct quantitative contribution of protozoa to fibre degradation. Other effects of defaunation, including the reduced ammonia levels and reduced pH of rumen fluid (eg

review Jouany, 1988), increases (Orpin & Letcher, 1984) or decreases (Kayouli *et al.* 1984; Ushida *et al.* 1986) of rumen fluid volume and fluid and solid phase retention times, effects on rumen fungi (eg Jouany *et al.* 1989), and direct toxic effects on bacteria of chemical agents applied to remove protozoa (Jouany *et al.* 1988), might all contribute to explanation of the effects of defaunation on fibre degradation. Apart from these effects, the simulations might give some indication of the effects of defaunation on NDF degradation in the rumen. Defaunation invariably reduced *in vivo* NDF or ADF degradation in the rumen (Veira *et al.* 1983; Kayouli *et al.* 1986; Punia *et al.* 1987; Ushida & Jouany, 1990; Ushida *et al.* 1990; Hsu *et al.* 1991), but the magnitude of the reduction differed widely. Defaunation results on *in sacco* NDF or ADF disappearance yielded more variable results, however, and both increases and decreases have been reported (review Jouany *et al.* 1989). Save the experiment of Punia *et al.* (1987), the *in vivo* experiments were performed with sheep at low intake levels (between 37 and 59 g DM/kg metabolic live weight). The simulations on dairy cattle at the lowest intake level (5.3 kg DM/d or 43 g DM/kg metabolic live weight) indicated a relatively high protozoal contribution to NDF degradation (17-21%). Thus, unless cellulolytic bacterial number or cellulolytic bacterial activity is increased to compensate protozoal fibre degradation, at such low intake levels defaunation might decrease fibre degradation in the rumen. At higher intake levels, protozoal contribution is reduced and consequently, defaunation might result in a less affected fibre degradation. Defaunation often resulted in an increase in the total number of bacteria (review Jouany *et al.* 1989), because protozoa prey on bacteria and both groups compete for available substrates. However, defaunation was shown to cause a shift in bacterial species present in the rumen. Kurihara *et al.* (1978) observed an increase in amylolytic bacterial numbers and a decrease in cellulolytic bacterial numbers, and defaunation resulted in a partly substitution of *Ruminococcus albus* by *R. flavefaciens*. The cellulolytic activity of the former is higher than that of the latter (Van Gylswyck & Labuschagne, 1971). Thus, an increase in cellulolytic bacterial activity due to removal of protozoa to compensate protozoal fibre degradation might not occur. Particularly with diets low in fibre and rich in starch and sugars, defaunation will likely reduce cellulolytic bacterial activity because of the lower pH of rumen fluid at these diets (review Jouany *et al.* 1988) and the detrimental effect of low pH values on cellulolytic bacterial growth (Russell & Dombrowski, 1980). Consequently, the higher protozoal contribution to fibre degradation predicted when dietary starch and sugar levels were increased at the expense of dietary NDF levels (Figures 2-4), will likely not be compensated for by an increased cellulolytic bacterial activity. With such diets, defaunation would be expected to decrease fibre degradation in the rumen to a larger extent than with roughage diets. Indeed, Ushida & Jouany (1990) observed a larger decrease due to defaunation in NDF degradation with a 65% lucerne hay, 30% barley diet (NDF degradation reduced from 41% to 28%) than with a 67% straw, 28% concentrate (beet pulp, soybean cake and groundnut meal) diet (from 51 to 45%). Equally, Ushida *et al.* (1990) reported a much

larger decrease in NDF degradation with a 75% straw, 19% maize diet than a 94% straw diet (reductions from 59 to 41% and from 64 to 59%, respectively). As stated before, other factors could influence these differences as well. Hsu *et al.* (1991) fed sheep 63% roughage, 37% concentrate diets and observed a significant increase in NDF degradation with defaunated animals, fed this diet supplemented with sodium bicarbonate, resulting in a higher pH of rumen fluid, compared with faunated animals fed the unsupplemented diet (42 and 34%, respectively). Next to the carbohydrate effects, N availability could exert an effect as well. Romulo *et al.* (1989) reported increases of the *in sacco* NDF digestibility on wheat straw diets resulting from defaunation, and showed interactions between defaunation and protein supplementation on fibre degradation. Again, simulations showed a decreased protozoal contribution to NDF degradation when dietary N levels were raised, save Diet RH (Figure 5C). Thus, in defaunated animals fed low N diets, cellulolytic bacterial activity should be increased to a higher extent to compensate for the loss in protozoal fibre degradation than on high N diets. However, as ammonia concentration in rumen fluid is almost invariable lower in defaunated animals (eg review Jouany *et al.* 1988), bacterial growth efficiencies will be reduced and such an additional compensation with low N diets is unlikely to occur.

In conclusion, the model provided a mathematical representation of the metabolic activities of amylolytic and cellulolytic bacteria and of protozoa in the rumen. It provided an integration of the effects of dietary composition and feed intake level on the different microbial groups represented in the model and the interactions between these groups, to give a coherent view of the contribution of protozoa to degradation of NDF in the rumen. Comparisons between predictions from the model and experimentally observed protozoal OM in the rumen showed general agreement for a range of dietary inputs. It was shown that, in general, increases in intake level reduced protozoal contribution, and substitution of roughages by concentrates increased protozoal contribution to NDF degradation in the rumen. These effects were further assessed by simulated changes in chemical composition of the diets. The predicted protozoal contribution, as influenced by the diet, provided possible explanations for the differences in rumen NDF degradation observed when animals are defaunated.

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GENERAL DISCUSSION

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The amount and type of nutrients ingested by ruminants generally differ considerably from those available for absorption, and ultimately for production. The microorganisms in the reticulo-rumen induce these differences by transformation of a variable portion of the ingested feed organic matter (OM), to microbial OM, and volatile fatty acids (VFA). Such changes are likely to influence the product (milk, meat, wool) and its composition to a significant extent (eg Thomas & Martin, 1988). The profile of nutrients available for absorption is the result of complex interactions which occur during fermentation. The main topic of the present study is mathematical modelling of the fermentation processes, to achieve the integration and quantification of these processes, with the long term aim of improving significantly, the level and efficiency of animal performance with respect to desired products and waste. The major objectives of the present Discussion, are to indicate how the models described in previous chapters help to increase knowledge on the transformation of ingested to absorbed nutrients and to predict these changes, and how this knowledge can be applied to describe feedstuffs with respect to their capability to support different types and levels of ruminant performance. In the first part of this Discussion, current feed evaluation systems and their limitations are discussed briefly and the basic assumptions and concepts for this Chapter are described. In the second part, which deals mainly with the scientific significance of this research, there is a discussion of the limitations, contributions and conclusions of the simulations, which were described in previous chapters, and how they lead to a better understanding of the integration and quantification of the fermentation processes. In the final part, which concentrates more on the practical significance of this research, the implications of these considerations to future feed evaluation systems are evaluated.

FEED EVALUATION SYSTEMS IN RELATION TO RUMEN FERMENTATION PROCESSES

Throughout this Chapter, reference to the phrase 'feed evaluation', infers the use of methods which characterize feedstuffs with respect to their ability to support different types and levels of animal performance (Beever & Oldham, 1986). Accurate feed evaluation systems facilitate management decisions on feed inputs relative to desired (milk, meat, wool), and undesired (respiratory, faecal and urinary) outputs. Presently, feed evaluation systems in most EC countries are based on metabolizable or net energy, and digestible crude protein or protein available for absorption (Van der Honing & Alderman, 1988) and are designed to meet fixed requirements for given outputs. The systems assign energy or protein values to feeds and assess the need of the animal in terms of these values, normally predicted using empirical equations, by a factorial approach. With this approach, the energy or protein requirements for

maintenance and production are established, and adjustments are made to accommodate for differences inherent in the feed (efficiency factors to convert metabolizable to net energy), and the physiological status of the ruminant (lactating, growing, pregnant). Limitations of these evaluation systems have been recognized and described (e.g. Beever & Oldham, 1986; MacRae *et al.* 1988; Webster *et al.* 1988; Gill *et al.* 1989; AFRC, 1991; Tamminga, 1992).

The first drawback of energy and protein evaluation systems is that they were developed independently. This offers only limited possibilities for consideration of the major energy-protein interactions within the rumen and the animal. For example, McCarthy *et al.* (1989) observed a significant interaction between energy and protein source on the glucogenic to non-glucogenic VFA ratio in rumen fluid. In the review of Oldham (1984), it was indicated that increases in the amount of dietary protein had no effect on dry matter (DM) digestibility at low intake levels, but the rise in dietary protein content increased digestibility of DM at high intake levels. Adjustments for the nature of available energy, are made by the use of estimates of the metabolizability (q; metabolizable energy/gross energy) of the diet, in empirical relationships to calculate the efficiencies of utilization of ME for maintenance, fattening and lactation. However, they fail to account for the effect of differences in protein available in the rumen or after absorption in the animal. Equally, present protein evaluation systems consider to a limited extent the effect of amount of ruminally digestible energy on microbial protein formed in the rumen, but not in relation to DM intake (DMI) level.

A second limitation of present feed evaluation systems, is that they do not allow a prediction of the response of product composition to changes in input. In the current payment systems, this is an important consideration since the quality of the products increasingly affects financial returns. The present requirement-based systems are not suitable to optimize the efficiency of the biological conversion of feed into the fat, protein and lactose components of milk, meat and wool, nor do they aim at limiting environmental pollution by feed residues from respiratory, faecal and urinary excretion. This shift from requirement to response needs more emphasis on the quantification and integration of the mechanisms which govern the response of animals to nutrients (AFRC, 1991). In order to do this, the conceptual framework of the system of interest must be quantitative, and hence mathematical models provide an appropriate way to represent concepts and mechanisms (Gill *et al.* 1989). This approach has been adopted in the models described in Chapter 1 and 4. Thus, rather than estimating microbial requirements for nutrients, the responses of microbes to changes in nutrient availability (viz. hexose, ammonia and amino acids/peptides) have been included in these models. Such a representation allows adequate predicted biological responses to nutrient availability ranging between microbial famine and feast (Bazin, 1981). Equally, the application of this type of mathematical models in plant physiology, has been successful in predicting responses to changes in nutrient availability, climatic conditions, etc. (Thornley & Johnson, 1990).

A third and final general assumption to be considered is the static character of present feed evaluation systems, and their inability to recognize the nature of the nutrients leading to energy or protein. Results of an experiment reported by Beever *et al.* (1988) illustrate this. ME intake, duodenal non-ammonia-nitrogen (NAN) flow, body and carcass energy, and protein and fat retention, were measured in growing beef steers fed early- or late-cut silage, supplemented with variable amounts of barley. ME intake and NAN flow were highest with the early-cut silage diet. Yet measured energy, protein and fat retention in the body and carcass were highest with the supplemented late-cut silage diet. Such variations could not be predicted within the framework of the UK protein and energy evaluation systems. The backgrounds and approaches adopted in various EC evaluation systems are comparable (Van der Honing & Alderman, 1988) and it seems justifiable to conclude that reservations concerning the UK systems, apply equally to other evaluation systems within the EC. Analogously, Beever & Oldham (1986) placed specific emphasis on avoidance of direct use of the q-factor in energy systems for dairy cattle. Explanation of such differences require recognition of the nature of ME and NAN of individual nutrients. With respect to energy yielding nutrients, major considerations should include the amount of glucose or glucose precursors and long chain fatty acids flowing out of the rumen, the amount and ratio of the different VFA produced and absorbed, and the effects of the absorbed nutrients on several hormones which direct the nutrients to production of milk and body protein and fat or other processes. The microbial efficiency parameters, true protein content, and individual amino acid composition should be recognized with respect to nitrogen (N) flow to the duodenum. Thus, in the developed models (Chapter 1 and 4), the level of outflow of individual nutrients was felt to be appropriate in view of the long term objective.

To summarize, the major emphasis within this Discussion is:

- the necessity of recognizing the metabolism of individual substrates, either within the rumen or available after absorption from the gastro-intestinal tract, instead of general energy or protein entities;
- the need to predict responses in product output and product composition, resulting from changes in feed input;
- an appreciation of the value of mathematical modelling in integration and quantification of the processes involved in transformation of ingested to absorbed nutrients, in order to broaden our knowledge concerning metabolic processes and ultimately to predict nutrient supply.

CONTRIBUTION OF RUMEN MODELS TO INTEGRATION OF FERMENTATION PROCESSES AND PREDICTION OF NUTRIENT SUPPLY

In this second part of the Discussion, the contribution of the mathematical models, developed and described in the present study, to the integration of fermentation processes and the prediction of nutrient supply will be discussed. Several rumen

models have already been described in literature (see Introduction). The models described in the present study are considered successful if they yield predictions which are better than predictions using existing models. Next to predictions of nutrient supply, the models can indicate the lack of data and gaps in current knowledge of rumen fermentation processes, which hamper correct predictions of nutrient supply. Thus, in this second part, comparisons between predictions of the models developed and existing models will be given, based on underlying (mathematical) concepts and data. The metabolic activity of the rumen microbial population is the major determinant of rumen fermentation. Mathematical representation of microbial metabolism has received considerable emphasis during the development of the present models. A discussion of the representations chosen in the present and previously developed models can be found elsewhere (Dijkstra *et al.* 1990).

On the mathematical representation of rumen fermentation processes

Given the basic assumptions described in the previous section, emphasis was placed on the mechanistic and dynamic characteristics of the models to be developed. For differences in underlying hypotheses and approaches between dynamic, mechanistic or static, empirical models, the reader is referred to textbooks (e.g. Thornley & Johnson, 1990). A model is only as good as the data and integration of information which is used in its development. Thus, the value of the models described in Chapter 1 and 4, depends on the underlying hypotheses as well as on data. Knowledge of the interactions between microorganisms and nutrients within the rumen, and interactions between animal factors and the fermentation processes, are still incomplete and subject to continuous experimentation. As a consequence, while the models may simulate the real fermentation processes well, for certain diets the lack of knowledge of all interactions involved will give rise to inappropriate responses. With research models however, the failure to respond accurately in certain situations is not a disadvantage, because it may indicate a direction for future research. Two examples of such inadequacies and the consequences for experimentation, have been described in the present study. Firstly, evaluation of the model described in Chapter 1 showed that VFA molar proportions were not predicted well, which was possibly related to a less accurate representation of individual VFA absorption rates (Chapter 2). An experiment was therefore conducted to establish the fractional absorption rates of acetic, propionic and butyric acid from the rumen, as affected by VFA concentration, pH, and rumen liquid volume (Chapter 3). The results clearly showed the importance of separation of the effects of VFA concentration and pH of rumen fluid, and the effect of rumen liquid volume on absorption rates. These findings could in turn be used to improve the prediction of VFA molar proportions. Secondly, model evaluation (Chapter 2) indicated the need for a more detailed representation of the metabolism of rumen protozoa. Both the level of organization and the lack of knowledge and data concerning microbial interactions, hampered a detailed representation (Dijkstra *et al.*

1990). Still, a model of the dynamics of protozoa in the rumen has been developed (Chapter 4), based on hypotheses on protozoal metabolism, whereas parameters were often assigned values on a priority basis. This mathematical integration of dietary and microbial characteristics provided a quantitative understanding of protozoal responses to changes in dietary inputs. Sensitivity analyses using this model, highlighted the need for quantitative data on bacterial engulfment rates, and protozoal maintenance requirements and death rates, and simulations (Chapter 4 and 5) substantiated and quantified the major impact of rumen protozoa in the transformation of ingested to absorbed nutrients. These examples confirm the frequently stated opinion that mathematical models can be a valuable tool in identifying gaps in current knowledge and in the design of experiments, which aim to elucidate metabolic processes (e.g. Kootsey, 1986; Gill *et al.* 1989; AFRC, 1991). The integrative nature of the developed models can be of major importance in enhancing scientific progress, in view of the fundamental role generally assigned to integrated research programmes (Kuhn, 1970).

The simulation of the fermentation processes in the rumen depends on the parameter values, hypotheses, and assumptions made within the model. In Chapter 1 and 4, emphasis has been placed on a full description of the complete model, to allow independent reconstruction, evaluation, and modification of the model and reproduction of the results. Often, parameter values have been assigned arbitrarily, and sensitivity analyses of the parameter values are required to establish the effects of a specific parameter value on the model results. In some cases, parameters can be estimated with high accuracy. For example, the calculated affinity constant of bacteria for uptake of ammonia (1.5 mM, calculated from data reported by Hume *et al.* 1970; Chapter 1), agreed favourably with the *in vitro* affinity constants of 0.2 mM for glutamine synthetase, which fixes ammonia at low concentrations, and 5 mM for glutamate dehydrogenase, which fixes ammonia at high concentrations (Baldwin & Koong, 1980). Increasing or decreasing this parameter by 50% hardly affected nutrient supply (results not shown). Yet different optimum levels of ammonia concentration for maximal microbial N synthesis rate have been suggested by different researchers (Stern & Hoover, 1979). This disagreement concerning optimum levels may at least be partly solved if ammonia requirements were to be replaced by responses to ammonia levels (see later in this section). However, in contrast with glutamate dehydrogenase, ammonia fixation via glutamine synthetase, requires ATP (Brown *et al.* 1974). Hence microbial growth at relatively low ammonia concentrations is likely to be less efficient energetically. Within the structure of the model, low ammonia concentrations increase the amount of hexose used for non-growth functions (Eq. 9.11 and 10.5 in Chapter 1). This is of particular importance in view of the competition between protozoa and bacteria in the rumen. In Chapter 4 it was shown that decreases in N availability resulted in competitive advantages for the protozoa, with a further negative effect on the efficiency of microbial growth. The general increase in protozoal OM in response to decreased N availabilities, agreed

qualitatively with the very few experimental data available. Thus, in this specific case, the structure of the model is more important in determining the responses to certain inputs than the parameter values and this seems to be a general feature in mechanistic models, though dependent on model objective (Thornley & Johnson, 1990). Quantitative estimation is important if the model is aimed at the determination of parameters, often because they are not at all measurable.

Model results appeared to be highly sensitive to some parameters, particularly to those related to protozoal metabolism (Chapter 4), affecting the simulated survival of *microorganisms*. The possibilities and problems of coexistence of microbial groups within a system has been encountered frequently (e.g. De Freitas & Fredrickson, 1978). The high sensitivity to these protozoal metabolism parameters probably reflect an oversimplified representation of the microbial interactions, including the factors and mechanisms directly related to maintenance, death and engulfment rate. Thus, selective protozoal engulfment of bacteria and other protozoa (Coleman, 1986), and differences in basal maintenance requirements (Russell & Baldwin, 1979) or affinity constants (Russell, 1984), have not been represented due to a lack of (quantitative) knowledge. Inclusion of these features would be expected to allow much more stable results (De Freitas & Fredrickson, 1978) and could decrease the sensitivity to protozoal metabolism parameters as well. Hence, even the high sensitivity to these parameter values showed the significant influence of the assumptions in the model and structure of the equations.

Driving variables to the models

Within the structure of the models in Chapter 1 and 4, the diet and dietary characteristics are major inputs. Other driving variables are pH, time below critical pH for optimal NDF degradation, passage rates, and (only for the Chapter 1 model) the fraction of protozoa in the amylolytic bacterial pool. These latter input variables are all, to a certain extent, influenced by the rumen fermentation processes, as will be explained in this section. Hence, the necessity of defining these variables before the start of the simulations, generally represents a limitation to the models.

The fraction of protozoa in the amylolytic bacterial pool was set at 0.35, and was decreased or increased with decreases in pH or increases in dietary lactate levels, respectively (see Chapter 1 for full explanation). This representation is a considerable simplification of the complex rumen microbial relationships. It ignores other effects on the ratio of bacteria to protozoa (Chapter 4), such as the different effects of N characteristics (Hino & Russell, 1987), and of passage rate values (Crawford *et al.* 1980). Thus, the necessity of representing the interactions between protozoa and bacteria in more detail became obvious (Chapter 2) and this has been subsequently addressed in Chapter 4. The more detailed representation of protozoal metabolism allows a prediction of the effects of the protozoa on the supply of nutrients, with specific emphasis on the recycling of microbial matter within the rumen, without the need to quantify certain protozoal aspects in the input of the model. As such, the

model in Chapter 4 is a considerable improvement on most of the previous rumen models, which did not account for protozoal metabolism effects (Baldwin *et al.* 1977; Beever *et al.* 1981a; Baldwin *et al.* 1987b), as well as on models which did include protozoal effects. Reichl & Baldwin (1976) included protozoal metabolism within their linear programming model of the rumen, but they had to specify the protozoal yields in the input. The rate of microbial catabolism was included in the model of France *et al.* (1982), but this rate was too low (cf. Leng & Nolan, 1984) for recycled microbial N to be of nutritional significance. In the Cornell energy and protein evaluation system, protozoal activity has been accounted for by a fixed decrease in the theoretical maximum growth yield (from 0.5 to 0.4 g microbial DM/g carbohydrate fermented; Russell *et al.* 1992). Thus, the model presented in Chapter 4 allows the prediction of a variable amount of recycling of microbial matter within the rumen on a variety of diets, without fixed settings of the variables related to this recycling. The validity and significance of this recycling due to protozoa, will be discussed later in this section.

The expected pH of the fluid has to be specified also. The importance of the pH on fibre degradation, and on VFA and ammonia absorption, is often acknowledged (see Chapter 1). It is obvious that the pH of rumen fluid is not an independent driving variable, but rather the result of the acids formed during fermentation of ingested nutrients, and the amount of buffering entities within the rumen. Some of these are predicted by the model itself, particularly VFA and ammonia concentration. However, the rate of absorption of VFA (and ammonia) depends on pH as well (Chapter 3), demonstrating the dynamic, integrated character of these relationships. Briggs *et al.* (1957) showed that while rumen pH was closely related to VFA concentration, even a common regression line of rumen pH on rumen VFA concentration with similar regression coefficients did not necessarily fit different diets. They suggested that the relationship between pH and VFA concentration might be considerably modified by variations in salivary secretion, which contributes to the bicarbonate and phosphate in the rumen, and the concentration of ammonia in rumen fluid. In contrast with bicarbonate, phosphate is of little value as a buffering agent in rumen fluid, but both phosphate and bicarbonate flowing with saliva neutralize acids in the rumen (Counotte, 1981). Tamminga & Van Vuuren (1988), using 244 data points in a pH range of 5.2 to 7.0, obtained a regression of pH on VFA concentration with a coefficient of determination (r^2) of 0.71. In the updated version of the model of Baldwin *et al.* (1987b), Argyle & Baldwin (1988) applied an equation to predict rumen pH by VFA and lactate concentration of rumen fluid, but did not give any details on the data set used to derive the equation or the accuracy of the fit. Subsequently, Argyle (1990) applied the regression equation of Briggs *et al.* (1957) to several data sets. He showed that individual r^2 were high (between 0.82 and 0.98), but also that the slopes and intercepts of each data set were different, in agreement with the original analysis of Briggs *et al.* (1957). When diets were fed to which phosphate or bicarbonate had been added, the slopes of the regression equation decreased, which

indicated the reduced sensitivity of changes in VFA concentration when buffers are included in the diet. In contrast with the updated Baldwin *et al.* (1987b) model, which included a fixed pH prediction equation, Argyle (1990) concluded that the equation to be used in the model should be taken from the actual diet fed or a diet as similar to it as possible. The inherent buffering capacity of the feed has been reviewed recently (Van Soest *et al.* 1991). This buffering capacity depends mainly on the cation exchange capacity of the fibre, which is the ability of the fibre to bind metal ions on its surface and to exchange these for hydrogen ions when the pH falls, rechanging when new cations become available. In this respect, both the size of particles ingested and the comminution and passage rates of particles are important, as well the cation exchange capacity of the individual feedstuffs in the diet. Considering the above mentioned factors, prediction of the pH of rumen fluid seems to require correct predictions of VFA, ammonia, and in certain circumstances lactate concentration; cationic exchange capacity of the fibre, and the concentration of fibre in the rumen; and particle size dynamics. While the prediction of VFA concentration, using pH as a driving variable, was satisfactory, that of ammonia concentration was not (Chapter 2), and the models completely ignored the effects of particle size or cation exchange capacity. Therefore, in the simulations, the pH had to be adopted from the observations of the actual diet fed or a similar diet, a conclusion which has been drawn previously (Argyle, 1990). Besides, the models in the present study assume steady state situations and do not allow for the diurnal variation in the pH of rumen fluid. The consequences of this assumption will be discussed later in this section. To summarize this section on pH of rumen fluid: the need to specify pH as an input factor represents a limitation to the model, but probably is not a disadvantage when compared with other models of rumen fermentation.

Analogous to the input of pH values to the model, fractional passage rate values were also adopted from the actual or similar diets. If unavailable, the regression equations of Owens & Goetsch (1986) were used. Even though these equations were based on a large dataset, the r^2 obtained was generally low. These equations predicted the fractional passage rate from a knowledge of DMI relative to body weight and from the proportion of concentrate in the diet. Similar empirical equations have been developed previously (Shaver *et al.* 1986; equations mentioned in Sniffen *et al.* 1992). However, in existing rumen models, passage rates either have to be specified between diets (France *et al.* 1982; Baldwin *et al.* 1987b) or are calculated using empirical regressions which require input specification of particle size or modulus of fineness factors (Baldwin *et al.* 1977; Black *et al.* 1981; Faichney *et al.* 1981). Though frequently applied, the low predictability of all of these equations presents a problem. Of more significance for research models is the fact that such empirical equations ignore the mechanisms of fluid and particle passage and their interactions with degradation through rumination and microbial activity. Generally, it is assumed that particles have to be reduced in size in order to leave the rumen (e.g. Ulyatt *et al.* 1986). Recently however, it was suggested that it was not the size of

particles *per se* which determines the disappearance rate, but rather the density or functional specific gravity (Kennedy & Murphy, 1988; Kaske *et al.* 1992). This, in turn, is influenced by the rate of hydration and the presence of air and gas spaces, resulting from microbial activity and the extent of degradation. Both the quantification of the main physiological processes, and knowledge of the external factors affecting these processes are limited (Faichney *et al.* 1989). The greatest progress in elucidating these processes will most probably be made by considering all of the mechanisms simultaneously. The models described in the present study could contribute to such an integration by providing a quantitative framework of the microbial degradation processes.

Finally, an important input for all rumen models is the amount of feed intake. Prediction of *ad lib* intake is a complex procedure which requires understanding of the processes of digestion and metabolism within the animal, as well as of environmental influences (Gill & Beever, 1988; AFRC, 1991). Often, prediction of intake has been based on empirical equations, which relate intake to variables such as live weight of the animal, milk yield, feed characteristics, etc. The accuracy of these predictions was satisfactorily when diets similar to those used to derive the parameters were fed, but for other diets or in other herds, voluntary intake was not predicted well (Neal *et al.* 1984; Elsen *et al.* 1988). Such findings suggest that the regression coefficients are applicable for only a limited range of diets and situations, and that adjustments are needed to cover specific dietary or environmental situations. Equally, it appears that a more complex representation of the factors controlling feed intake is required than can be provided by empirical equations. Many theories on this subject have been described, including physic, chemostatic and thermostatic control of feed intake, and general theories on a desired feed intake of an animal, providing enough feed resources to achieve its objectives, have been proposed (Forbes, 1986). Recently, a somewhat different chemostatic theory has been proposed, in which voluntary feed intake is assumed to be reached at a level where the ratio between energy benefits and oxygen costs to the animal is optimal (Ketelaars & Tolkamp, 1991). Mechanistic approaches to voluntary feed intake are concerned with the different mechanisms controlling feed intake and their interactions. Progress in predicting DMI is to be achieved by integration of the digestive and metabolic processes within the rumen, the animal and the environment (Gill & Beever, 1988; AFRC, 1991; Dijkstra & Makkink, 1993). The models in the present study could contribute to such an approach by predicting the metabolic activities of the microorganisms and the resulting fermentation products, providing estimates of nutrient availability, to be used in models of metabolism in the body. However, the models described in Chapter 1 and 4 work in a steady state and do not predict patterns of VFA concentration in rumen fluid nor of the rumen pool size of degradable and undegradable fibre, though both may be feedback signals to voluntary feed intake. France *et al.* (1982) obtained reasonable agreement between rumen model output and data on sheep fed different diets frequently, but considerable discrepancies occurred when discontinuous feeding

patterns were simulated. Baldwin *et al.* (1987b) included a much more detailed consideration of particle size and particle size reduction than France *et al.* (1982). Unfortunately, these authors did not validate aspects of discontinuous feeding against independent data, but results of simulations with once daily feeding were considered realistic. The inability of the models described in the present study to represent dynamic feeding behaviour, is illustrated in a comparison between observed and predicted rumen contents of NDF of the previously mentioned experiment of Robinson *et al.* (1987) with different DMI levels (Table 1). Though r^2 was high (0.995) the root Mean Square Prediction Error (MSPE; see Chapter 2 for definition) was 30% of the observed mean, and overall bias contributed 79% to the MSPE. The mean bias showed consistent underprediction of NDF content in the rumen, which can generally be rectified by a mean correction factor. Some error might be attributed to the calculation of mean NDF pool size using pooled samples which represent the average of 3 rumen evacuations (Robinson *et al.* 1987). However, the most likely reason for these differences, is the steady state nature of the model, ignoring the differences in passage rates of particles which have been subject to various stages of degradation. Large and/or light particles, newly arrived in the rumen, are likely not washed out until appropriate size and/or density of the particles is reached. These differences have been included in the model of Baldwin *et al.* (1987b). Thus, inclusion of a time-lag in the present models to accommodate for such behaviour of the particles would decrease the differences between observed and predicted NDF pool sizes. Such a time-lag would not be appropriate for VFA disappearance, because all VFA in the fluid are subject to clearance by absorption and passage. To a limited extent, this would apply to microbial OM in the rumen as well, though dependent on the distribution of microorganisms which are free in rumen fluid or attached to small and large particles and the rumen wall. Indeed, such a bias was not apparent for observed and predicted rumen bacterial pool size, calculated using DAPA as a bacterial marker, or VFA concentration on the same experiment (Chapter 2). In conclusion, representation of non-steady state situations probably requires additional information, particularly with respect to particle dynamics within the rumen.

Prediction of NDF, starch and sugar flow

A large amount of OM flowing to the duodenum consists of NDF. The capacity of the hindgut to degrade NDF is limited and the microbial matter synthesized from NDF degraded in the colon will not be absorbed (Van Soest, 1982). Therefore, it is important to optimize the degradation of fibre within the rumen if the utilization of fibrous feeds is to be maximized. Rumen degradation of NDF will be determined by inherent characteristics of the NDF in the feed (potential degradability and rate of degradation), the actual degradation rate in the rumen and the passage rate of NDF to the duodenum. The effects of passage rate and the consequences for voluntary feed intake have been described earlier. Rate of degradation will be affected mainly by feed characteristics as well as by the activities of the microbial population present. In this respect, two major aspects influencing microbial activity are the pH of rumen

Table 1. *Comparison of experimental observations (obs) with model predictions (pred) of rumen content of Neutral Detergent Fibre (NDF) for cows fed diets at different intake levels*

	Intake level (kg DM/d)									
	5.3		9.2		13.1		17.1		21.0	
	obs	pred	obs	pred	obs	pred	obs	pred	obs	pred
NDF pool (kg)	1.58	1.24	2.35	1.84	3.49	2.46	4.05	3.07	6.02	4.31

Observations from Robinson *et al.* (1987).

fluid and N availability. The latter will be discussed later. Whilst the effect of rumen fluid pH on NDF degradation has generally been recognized, its representation in mathematical models has received only limited attention. In the present model, the representation of the pH depressing effect was adequate (Chapter 2). Using the model of Baldwin *et al.* (1987b) it was recognized that the prediction of NDF degradation on high concentrate diets was not satisfactorily. In the updated version Argyle & Baldwin (1988) included the effect of pH on fibre degradation. While based on virtually the same sources, the form of the equation to adjust for pH in their model differed from the equation in the model described in Chapter 1. Reducing the pH below 5.7 would decrease NDF degradation to zero in the model of Argyle & Baldwin (1988), whereas in the present model, there would still be some degradation, albeit at a low level. Model simulations described in Chapter 5, indicated that NDF degradation in these circumstances could be attributed largely to the activities of the (relatively few) protozoa remaining in the rumen at such low pH values. In contrast with the representation chosen by Argyle & Baldwin (1988), Terry *et al.* (1969) and Hoover *et al.* (1984) observed *in vitro* NDF disappearance, after 24 h and pH of 5.5, which ranged between 0 and 31% of the disappearance at pH 6.8 or 6.5. Equally, unlike the present models, utilization of the equation derived by Argyle & Baldwin (1988) could not reproduce the initial small decrease in NDF degradation at pH 6.2 observed *in sacco* by Grant & Mertens (1992). Black *et al.* (1981) in their model recognized the decline in fibre degradation which occurred when the diet contained considerable amounts of starch or soluble sugars. They included a dimensionless parameter, allowing the fibre degradation rate to be changed in the presence of starch and sugars, and suggested that the value of this parameter could be calculated from actual *in vivo* or *in vitro* studies. Obviously, this approach is of less value if a prediction of the degradation of fibre in the rumen is required. In the approach adopted in the rumen submodel of the Cornell evaluation system, the effect of pH on microbial growth yield is predicted from the NDF content of the ration (Russell *et al.* 1992). Although attractive because of its simple calculation, such an approach ignores the different effects of feed intake level and degradation rate of the non-NDF components (particularly starch) of the diet. For example, both ruminal pH and NDF

degradation tended to be lower when barley replaced corn in diets with almost equal NDF contents. Effects were more pronounced in the case of an increase in the amount of barley than of corn in the diet (DePeters & Taylor, 1985; McCarthy *et al.* 1989). Overall, the representation of NDF degradation within the rumen, and particularly of the depressant effect of pH on NDF degradation as advanced in the present study, is a feasible approach to obtain correct predictions of rumen NDF degradation and has a considerable advantage over existing models. However, as discussed previously, the need to specify pH as an input factor instead of being calculated during simulations is a disadvantage, probably preventing further application.

Unlike NDF which escapes rumen fermentation, nonstructural carbohydrates can be enzymatically digested in the small intestine. Postruminally digested starch can be used more efficiently for milk and body synthesis than can that degraded in the rumen (Baldwin *et al.* 1980; Nocek & Tamminga, 1991). However, even substantial passage of nonstructural carbohydrates did not result in net glucose absorption from the portal-drained viscera (PDV). Feeding high concentrate diets resulted in net absorption of glucose by post-stomach tissues, but net uptake of glucose by stomach tissues increased as well, resulting in a small net utilization of glucose by PDV (review Huntington, 1990). The possible significance of postruminally absorbed glucose might be a sparing effect of the utilization of other nutrients (amino acids or glucose synthesized endogenously) by the gut. In previous modelling exercises, and in the present study, the comparison of observed and predicted starch and sugar outflow to the duodenum has received less attention, because *in vivo* outflow data of sugars and starch has been reported less often than NDF flow, and an inadequate description of diets hampered a comparison. Validation of the model of France *et al.* (1982) against outflow of α-hexose reported by Beever *et al.* (1981b) (4 dietary treatments) indicated qualitative agreement between diets, but considerable bias occurred (MSPE 79% and bias contributing 74% toward MSPE). The model of Baldwin *et al.* (1987b) was validated against data reported by Sutton *et al.* (1980) and Sutton (1985), with 4 diets containing different amounts of corn or barley. A MSPE of 30% (with contribution of the random variation about the regression line of 54%) can be calculated. In the present model (Chapter 1), both the predicted and observed passage of starch to the duodenum were much higher for dairy cattle fed high concentrate diets, based on maize, than barley based diets (McCarthy *et al.* 1989; Chapter 2) at intake levels exceeding 20 kg DM/d. Duodenal starch flow was overpredicted for 50% roughage, 50% concentrate diets (DMI 18.3 and 19.4 kg/d; Robinson & Kennelly, 1990), but this could possibly be attributed to the choice of marker to estimate duodenal flow (Chapter 2). Goetsch & Owens (1986a, b) fed high concentrate or high roughage diets (more than 80% concentrate or roughage, respectively) with low or high N levels to beef steers (DMI 3.8 - 4.0 kg/d). Observed starch outflow was low with the high roughage diets (160 and 96 g/d with low and high N level, respectively), and model predictions agreed favourably (172 and 99 g/d,

respectively; using the model from Chapter 4). In contrast, for high concentrate diets, a (non significant) higher starch outflow with the high N level (680 g/d) than with the low N level (604 g/d) was observed, whereas the model predicted the reverse (505 and 595 g/d with high and low N level). The reason for such discrepancies is not clear. Validation against starch outflow data on these 4 diets reported by Goetsch & Owens (1986a, b) yields a MSPE of 23% of the observed mean and 57% of MSPE attributed to the disturbance proportion. Generally, a variable proportion of the starch outflow could be of microbial origin. While all previous rumen models have assumed a fixed amount of carbohydrates in the rumen microbial DM, the present models assumed a variable proportion, related to microbial group (protozoa, amylolytic or cellulolytic bacteria), and dietary composition (see Dijkstra *et al.* 1990 and Chapter 1 and 4). Unfortunately, no adequate datasets are available to validate the assumptions in the model with respect to the content of microbial storage polysaccharides, but responses to N and carbohydrate availability were qualitatively in line with *in vitro* observations (Chapter 2). The representation chosen in the present model showed that variation in microbial composition could be represented relatively simply. The amount of storage material formed could significantly influence the microbial efficiency, commonly expressed as a unit of microbial N per unit of OM degraded or fermented (see later in this section).

The outflow of soluble sugars to the duodenum is hardly ever measured *in vivo*. It is generally assumed that soluble sugars are almost completely removed in the rumen (Van Soest, 1982). In the models, some escape of soluble sugars to the duodenum will inevitably occur, because the simulated pool of soluble sugars is prone to uptake by microorganisms and to wash out from the rumen. Beever *et al.* (1981b) fed chopped or pelleted Italian ryegrass (water soluble carbohydrate content 207 and 212 g/kg DM, respectively) and chopped or pelleted timothy (water soluble carbohydrate content 41 and 64 g/kg DM, respectively) to sheep. Despite the four times higher intake of water soluble carbohydrates of the Italian ryegrass diet, there were no differences between diets in quantities of water soluble carbohydrates flowing to the duodenum (between 5.0 and 6.9 g/d). Predictions of steady state outflow of sugars for these 4 diets by the model of France *et al.* (1982) were too high (ranging between 10.3 and 16.2 g/d; MSPE of 122%). A daily outflow of 3.2 g soluble carbohydrates could be calculated, assuming a constant, basal level of soluble carbohydrates in the rumen fluid, amounting to 0.35 g/l (Clapperton & Czerkawski, 1969), a rumen volume of 5 l (Siddons *et al.* 1985) and fluid passage rates of 0.076 /h (Beever *et al.* 1981b). However, due to the feeding pattern in the experiment of Beever *et al.* (1981b) (twice daily), a peak in the sugar concentration in rumen fluid immediately after eating could be expected (Clapperton & Czerkawski, 1969) and could account for the remaining sugar outflow. Thus, there is some indication that a certain minimum amount of sugars will pass to the duodenum, even though it is at a low level. In conclusion, while starch and sugar outflow could not be sufficiently validated, the simulations suggest that predictions are at least as good as predictions

with existing models. The present models however include representation of variations in microbial composition, as observed *in vivo* and *in vitro*, and thus allows evaluation of this variation with respect to microbial efficiency.

Prediction of non-ammonia nitrogen (NAN) flow

Considerable attention has been paid to the flow of N fractions to the duodenum, and factors which affect this flow have been reviewed (e.g. Robinson & Tamminga, 1984; Nocek & Russell, 1988; Polan, 1988; Mackie & White, 1990; Clark *et al.* 1992). The composition of the N flow to the duodenum is of importance for the ultimate production of the animal. A part of the feed protein which escapes rumen fermentation, will not be degraded in the small intestine (Van Straalen & Tamminga, 1990). Equally, nucleic acid N and N in the cell walls of bacteria will not be utilized by the animal and will be excreted in faeces or urine. Of course, the amino acid (AA) composition of the N flowing to the duodenum is also of importance. With respect to these considerations, the amount and composition of AA available for absorption seems to be appropriate, analogously to the ileal digestible AA systems adopted for pigs (Low & Zebrowska, 1989). Therefore, it is important to differentiate the AA flow into those of dietary and of microbial origin. Differences in passage of individual AA may not be very pronounced, since microbial protein generally supplies a large quantity of total AA that passes to the small intestine, and since the AA composition of bacteria is constant (Storm & Ørskov, 1983). However, from a summary of bacterial samples of animals fed 61 dietary treatments, Clark *et al.* (1992) reported that bacterial AA composition (expressed as units of individual AA per unit total AA) was not constant. A portion of this variation was attributed to variations in techniques for isolation of the bacteria, and for measurement of bacterial composition. The digestibility and AA composition of protozoa differs from that of bacteria (Czerkawski, 1976). The protozoal AA profile has increased proportions of lysine and glutamate, and decreased proportions of glycine and alanine. However, the outflow of protozoal N is generally assumed to be low (see Chapter 4; the model predictions of the contribution of protozoal NAN to total duodenal microbial NAN flow on a range of diets varied between 6 and 18%) and such differences between bacterial and protozoal AA composition will probably not affect duodenal AA composition profoundly. Some support for this suggestion are the results of a defaunation study, in which the duodenal AA composition of faunated and defaunated animals did not differ (Veira *et al.* 1983). On the other hand, Onodera & Koga (1987) studied first limiting AA in goats, and their results with faunated (histidine and methionine as first and second limiting AA) and defaunated animals (histidine and lysine as first and second limiting AA) suggested that protozoal AA might affect total duodenal AA composition. Besides, the AA profile of dietary protein potentially escaping fermentation can be different from the AA profile of the ingested dietary protein (Sniffen & Chalupa, 1990).

The total amount of NAN flowing into the duodenum was predicted with high

accuracy using the model developed in the present study (Chapter 2) as well as using existing rumen models or current protein evaluation systems (e.g. Ramangasoavina *et al.* 1991). However, the prediction of microbial and non-microbial components of NAN flow was less satisfactory. At least some part of the error can be attributed to differences in techniques (particularly to the bacterial and/or protozoal isolation procedures and the markers applied), as indicated by the much higher variation in measurement of microbial NAN flow than total NAN flow (Oldham & Tamminga, 1980). In mechanistic models, an under- or overprediction of microbial biomass in the rumen will result in under- or overprediction respectively, of feed N degradation. Consequently, any error in the prediction of microbial NAN flow will be compensated for by the predicted non-microbial NAN flow, resulting in relatively correct predictions of total NAN flow. Ramangasoavina *et al.* (1991) evaluated the INRA protein evaluation system (Vérité *et al.* 1987), the Cornell feed evaluation system (Russell *et al.* 1992), and the model of Baldwin *et al.* (1987b) with respect to microbial and non-microbial NAN flow. From the underpredicted microbial NAN flow at high total NAN flows to the duodenum, they concluded that the models needed an improved representation of recycling of urea to the rumen, and a better quantification of the effect of differences in DMI. The same conclusion with respect to N recycling has been reached previously by Beever *et al.* (1981a). The model of Baldwin *et al.* (1987b) represented recycling of N by saliva only, whereas the other models did not include N recycling. Equally, none of the present protein evaluation systems take the amount of N recycled to the rumen into account in a quantitative manner. Though such low N diets would probably decrease animal performance, in view of the aim of reducing N excretion to the environment, this could become an important aspect of diet evaluation. In the models developed in the present study, equations to represent protein and urea entering the rumen with saliva and through the rumen wall have been included (Chapter 1). It was recognized that this representation largely ignored the interaction between animal and rumen factors in the urea concentration of the blood, possibly giving rise to inadequate predictions of ammonia concentrations in rumen fluid. Yet the prediction of NAN outflow on diets was satisfactorily where total NAN flow exceeded N intake by up to 20% (Chapter 2). Thus, these results indicated that N recycling was adequately represented and as such, the present model is a considerable improvement on previous models, particularly considering environmental constraints. Moreover, there was no tendency to underestimate microbial NAN flow at high total NAN flows as reported by Ramangasoavina *et al.* (1991). Rather, the predicted microbial NAN flow tended to be higher than observed, with a MSPE of 37% of the observed mean and 69% of this error was attributable to the deviation of the slope to unity (Chapter 2). Russell *et al.* (1992) reported a high correlation between observed and predicted bacterial N flow (r^2 = 0.88; cf Chapter 2, r^2 = 0.86) using the Cornell protein evaluation system. However, the data they used for validation group in two clusters of points (one high N flow group with dairy cattle and one low N flow group with steers) that are widely separated, and analysis of each

individual group would yield different results (Glantz & Slinker, 1990), likely increasing bias severely. Overall, the flow of NAN to the duodenum was predicted accurately by the present model. The representation of recycling of N to the rumen is an improvement over earlier models in that NAN flow on low N diets is predicted satisfactorily. Equally, the model of Chapter 4 contained in addition to essential microbial features included in existing models, a detailed consideration of protozoal metabolism. This allows evaluation of concepts about protozoal metabolism and offers opportunities to predict the AA profile of duodenal NAN flow.

Prediction of efficiency of microbial synthesis

The factors affecting microbial efficiency have been frequently discussed (e.g. reviews Robinson & Tamminga, 1984; Sniffen & Robinson, 1987; Hoover & Stokes, 1991; Clark *et al.* 1992). A number of these factors, including feed intake, roughage:concentrate ratio, and source of carbohydrates and protein, can be evaluated using the models. Other factors, such as the fat content of the diet and availability of micronutrients, have not been included in the models and it is assumed that a shortage or overabundance will not occur in the diets used in simulations. Efficiency figures vary widely and opposite effects of the factors referred to, have been reported (see reviews mentioned). The present protein evaluation systems adopted fixed efficiency figures. While this would probably affect the predicted total flow of protein to the duodenum to a much smaller extent than prediction of the protein of microbial and non-microbial origin, the importance of a correct prediction is clear when AA profiles have to be predicted. If N availability in the rumen is low, energy spilling reactions can occur (e.g. review Harrison & McAllan, 1980) and such an uncoupled fermentation has been represented in the developed models (Chapter 1 and 4). There is much controversy concerning the N requirement for maximum synthesis of microbial protein and degradation of OM in the rumen (see e.g. Stern & Hoover, 1979; Hoover & Stokes, 1991). In attempts to elucidate these differences, it is important to appreciate the dynamic nature of the fermentation processes affecting concentrations of ammonia and AA/peptides in the rumen. For example, low concentrations could limit microbial growth, but could also be the result of a very efficient growth as well, as indicated by the high efficiencies and low ammonia concentrations generally found in defaunated animals (Jouany *et al.* 1988). The present models can enhance understanding, in that their predictions are based on underlying mechanisms, thus accounting for different factors such as level of intake, source of energy and N, etc. In Table 2, simulation results of the responses to changes in N availability of the high roughage (Diet R) and high concentrate (Diet C) diets from Chapter 4 are presented, using the model described in that Chapter. In these simulations, the concentration of ammonia was fixed at the predicted level (11.3 and 9.9 mM for Diet R and C, respectively) or decreased to 5 or 1 mM. The results showed that a decrease in ammonia concentration resulted in an increase in feed OM outflow and a decrease in microbial OM and total and microbial NAN flow,

Table 2. *Model predictions of effect of changes in ammonia concentration on ruminal carbohydrates apparently (ADCHO) or truly (TDCHO) digested and OM apparently (ADOM) or truly (TDOM) digested, microbial NAN outflow, and efficiency of microbial synthesis (E) for cattle*

	Diet					
	R	R5	R1	C	C5	C1
TDCHO (% of intake)	66.5	65.8	64.3	66.5	65.9	64.2
ADCHO (% intake)	64.6	63.8	62.5	62.3	61.7	59.8
TDOM (% intake)	64.6	63.9	62.4	61.2	60.6	59.1
ADOM (% of intake)	50.0	49.9	49.6	47.1	46.8	46.1
Microbial NAN flow (g/d)	261	248	229	205	198	181
E (g N/kg TDCHO)	34.0	32.6	30.9	29.0	28.3	26.4
E (g N/kg ADCHO)	35.1	33.6	31.8	31.0	30.2	28.4
E (g N/kg TDOM)	27.3	26.2	24.8	24.1	23.5	22.0
E (g N/kg ADOM)	35.3	33.5	31.2	31.3	30.4	28.2

For details of diets, see Chapter 4; Diet R, 90% roughage, 10% concentrate diet; Diet R5, same as Diet R, with ammonia concentration fixed at 5 mM; Diet R1, same as Diet R, with ammonia concentration fixed at 1 mM; Diet C, 30% roughage, 70% concentrate diet; Diet C5, same as Diet C, with ammonia concentration fixed at 5 mM; Diet C1, same as Diet C, with ammonia concentration fixed at 1 mM.

and also decreased efficiency of microbial synthesis. Qualitatively, these results are in line with *in vivo* data showing diminishing returns of supplementary urea (Hume *et al.* 1970). The reason for the decrease in efficiency is an increased amount of energy used by bacteria for non-growth purposes and a relative larger protozoal portion of total microbial biomass in the rumen, which was described in Chapter 4. Of course, supplementation of diets with either urea or protein will inevitably decrease the concentrations of NDF, starch and lipid in the diet and an example is given in Table 3. In these simulations, it was assumed that equal amounts of DM in the diet were substituted by a pure, qualitative equivalent protein, raising or decreasing dietary protein content by 15%, and that DMI was unchanged. Predicted microbial efficiency (g N/kg TDOM or g N/kg ADOM) was decreased slightly when dietary protein content was greater than the control diet. The reason for this decrease in response to increased dietary protein-N is that NDF, starch and sugar contents of the diet were decreased, and protein fermentation yields less ATP than carbohydrate fermentation (Tamminga, 1982a). This decrease was not fully compensated for by the improved microbial energetic efficiency (g N/kg TDCHO or ADCHO) which resulted from increased N levels. With diets containing less N, supplementary protein increased this energetic efficiency to a much larger extent and this resulted in an increase in efficiency (g N/kg OM digested). However, with Diet C, there were also changes in the composition of microbial biomass. With a decrease in the N content of Diet C, the

availability of energy for protozoa was increased and this resulted in higher fractional death rates of protozoa (see Chapter 4). This decrease in protozoal biomass decreased microbial turnover as well. Consequently, microbial NAN outflow on Diet C- was higher than Diet C, and efficiency (g N/kg TDOM or ADOM) hardly changed. From Table 3 and taking into account the above considerations, it is clear that the dietary N content which would result in the highest efficiency differs between diets. Indeed, adding protein to the diet has been found to decrease microbial efficiency (g N/kg OM digested) at high dietary N levels (Veira & Ivan, 1982; Lu *et al.* 1982), but increased efficiency at low N levels (Veira & Ivan, 1982). The results presented in Table 2 and 3 have been performed at high feed intake levels. At low intake levels, the balance between all the factors affecting the response to changes in dietary N levels would determine the dietary N level at which efficiency is highest.

Table 3. *Model predictions of effect of substitution of diet ingredients by protein on ruminal carbohydrates apparently (ADCHO) or truly (TDCHO) digested and OM apparently (ADOM) or truly (TDOM) digested, microbial NAN outflow, and efficiency of microbial synthesis (E) for cattle*

	Diet					
	R-	R	R+	C-	C	C+
TDCHO (% of intake)	66.1	66.5	66.6	66.6	66.5	65.9
ADCHO (% intake)	64.2	64.6	64.6	62.3	62.3	61.7
TDOM (% intake)	64.0	64.6	64.5	61.9	61.2	59.8
ADOM (% of intake)	49.7	50.0	50.1	47.1	47.1	46.3
Microbial NAN flow (g/d)	256	261	260	207	205	197
E (g N/kg TDCHO)	31.9	34.0	35.6	27.8	29.0	29.5
E (g N/kg ADCHO)	32.9	35.1	36.5	28.2	31.0	31.5
E (g N/kg TDOM)	27.0	27.3	27.2	24.1	24.1	23.7
E (g N/kg ADOM)	34.8	35.3	35.1	31.3	31.4	30.6

For details of diets, see Chapter 4; Diet R, 90% roughage, 10% concentrate diet; Diet R-, same as Diet R, with protein and non-protein content decreased and increased by 15%, respectively; Diet R+, same as Diet R, with protein and non-protein content increased and decreased by 15%, respectively; Diet C, 30% roughage, 70% concentrate diet; Diet C-, same as Diet C, with protein and non-protein content decreased and increased by 15%, respectively; Diet C+, same as Diet C, with protein and non-protein content increased and decreased by 15%, respectively.

From these simulations, it is clear that the definition of efficiency must be stated clearly to avoid confusion. For example, Stouthamer (1973) calculated that in the absence of preformed AA, protein synthesis would require 5.35 mol ATP per mol protein, while in the presence of AA this would be reduced to 5.0 mol ATP. In their review, Nocek & Russell (1988) stated that addition of protein to *in vitro* cultures or the substitution of urea by protein, increased growth yield to a much larger extent

than was expected from the theoretical calculations of Stouthamer (1973). However, Nocek & Russell (1988) defined growth yield as g bacterial DM per g carbohydrate. From Table 3 in Chapter 1, it is clear that the synthesis of protein from ammonia requires little extra energy *per se*, but significantly higher amounts of carbohydrates as carbon skeletons for microbial AA. Hence, the efficiency based on ATP values would change less drastic than the efficiency based on carbohydrate values. Hoover & Stokes (1991) calculated a correlation of 0.80 and 0.59 between the degradable protein content of feed and microbial efficiency (g N/kg CHO digested) from data using 7 continuous culture experiments or 7 trials with dairy cattle, respectively. These results agree with the improvement in microbial efficiency (g N/kg TDCHO or ADCHO) seen for increased ammonia concentration or dietary protein levels as predicted by the model (Table 2 and 3). Such differences would be different however, if the third measure of efficiency was applied, which is the unit microbial N per unit OM utilized. Thus, from the theoretical calculations of Stouthamer (1973), the synthesis of 1 gram of microbial protein from preformed AA requires 1.64 g hexose and 1 g AA (total 2.64 g OM), whereas synthesis from ammonia requires 3.00 g hexose and 0.19 g ammonia (total 3.19 g OM) (Table 3 in Chapter 1). The results in Table 3 showed that while energetic efficiency (g N/kg TDCHO or ADCHO) was always improved with increases in N availability, efficiency based on TDOM or ADOM was not necessarily improved, consistent with these considerations. Experiments in which dietary urea was replaced by protein often revealed a decrease in efficiency (g N/kg TDOM or ADOM) (Mercer *et al.* 1980; Ha & Kennelly, 1983; Ling *et al.* 1983; McAllan *et al.* 1988). However, in these experiments, the replacement of urea by protein was accompanied by a reduction in carbohydrate content (particularly starch), and consequently one would expect such a decrease in efficiency. To summarize, the simulations have highlighted the dynamics of microbial growth efficiency in the rumen, and indicate the need to consider the integration of factors influencing this efficiency.

Finally, the effects of feed intake and roughage:concentrate ratio can markedly affect microbial N synthesis and efficiency. This is illustrated in Figure 1. In this Figure, model predictions (using the model of Chapter 4) are presented for the diets described in Chapter 5, in which the dietary concentrate content ranged between 0 and 100% and DMI varied between 5.3 and 21.0 kg/d. The storage polysaccharide content of microbial DM was higher when the concentrate proportion was increased, and was reduced in the case of increased intake levels (Figure 1A). With the 100% concentrate diet at intake levels exceeding 15 kg DM/d there was a marked increase in polysaccharide content which was the result of the complete disappearance of cellulolytic bacteria, which were assumed not to contain any storage polysaccharides. Such differences would affect the efficiency of microbial N synthesis, because energy is used to synthesize a non-N containing compound. For low roughage diets fed at high intake levels, the microbial efficiency would be at its highest with respect to microbial composition. Unfortunately, there are no data available to validate such

differences as affected by dietary characteristics.

Theoretically, the turnover of microbial OM in the rumen will have a large effect on microbial efficiency as well. Turnover can largely be attributed to the activities of rumen protozoa (Leng & Nolan, 1984; Wallace & McPherson, 1987). Thus, changes in the amount and activities of rumen protozoa would affect turnover and ultimately the efficiency of microbial growth. Apart from high concentrate diets, the proportion of protozoa in the total microbial biomass was generally predicted to decrease with increasing intake levels (Figure 1B; Chapter 4). The effect on turnover within the rumen, however, was significantly affected by the roughage:concentrate ratio. Microbial turnover decreased when an all roughage diet was fed (from 0.96 to 0.57 /d at lowest and highest intake level, respectively), but increased when the all concentrate diet was simulated (from 0.54 to 1.18 /d at lowest and highest intake level, respectively). The diets in between showed intermediate values. The marked increase when high concentrate diets were fed, can be explained by the increase in protozoal OM as a proportion of total microbial OM and the rapid increase in fractional death rates of protozoa (Chapter 4). For high roughage diets, the increase in the fractional death rate does not compensate for the decrease in turnover which results from a decreased engulfment of microorganisms, which is in turn caused by a decrease in proportional protozoal OM. The large effect of protozoa on turnover of microbial matter in the rumen, and hence on microbial efficiency, could explain the generally large increase in microbial efficiency following rumen defaunation (Jouany *et al.* 1988). There are few data available to confirm such relationships. *In vitro*, approximately 90% of protein breakdown of bacterial cells was related to the presence of protozoa (Wallace & McPherson, 1987), and individual protein breakdown rates ranged between 0.05 and 0.30 /h. Van Nevel & Demeyer (1977) reported that 50% of microbial matter synthesized was degraded again. *In vivo*, recycling of microbial matter via the ammonia pool was 30 to 50% of the total ammonia flux in sheep (review Leng & Nolan, 1984). Aharomi *et al.* (1991) calculated that for a 75% concentrate diet, recycling to the ammonia pool contributed 22 to 62% of total microbial synthesis. However, recycling is not only via the ammonia pool, because protozoa partly incorporate engulfed bacterial AA, and bacteria can incorporate the AA released into rumen fluid by protozoa (see model description in Chapter 1 and 4). Recently, Firkins *et al.* (1992) calculated that in cattle, recycled microbial NAN was 90% when an 85% corn silage diet was fed at 4.7 kg DM/d, and this recycling decreased to 75% when the intake level was 10 kg DM/d. This decrease due to intake level on a high roughage diet qualitatively agrees with the results in Figure 1C.

The impact of recycling on microbial efficiency is large, as can be seen in Figure 1D, and considering its significance for microbial efficiency, the results highlight the need to quantify recycling in different dietary situations. Microbial efficiency is affected by a number of factors and their interactions, which hamper a rapid interpretation of results. At low intake levels, the predicted efficiency of high roughage diets was much lower than for high concentrate diets (Fig 1D). This is

mainly the result of the higher turnover rate (Figure 1C), and the lower ratio of bacteria free in fluid (amylolytic bacteria) to bacteria attached to particles (cellulolytic bacteria) with the roughage diets, whereas protozoal OM (per unit total microbial OM) was not different. Amylolytic bacteria were assumed to be washed out faster than cellulolytic bacteria, which in turn flow to the omasum more rapidly than protozoa. *In vitro*, the faster outflow has been shown to increase efficiency, because relatively less energy is used for non-growth purposes (e.g. Russell, 1984). *In vivo*, this relationship is not immediately apparent. Increases in efficiency with increases in fluid passage rates were observed by Harrison *et al.* (1975 and 1976), Kennedy *et al.* (1976) and Kennedy & Milligan (1978), but Chamberlain & Thomas (1980), Hadjipanayiotou *et al.* (1982) and Goetsch & Owens (1985) found either no relationship or even a decrease in efficiency. Other changes in microbial metabolism resulting from an increase in passage rate could have affected the final efficiency. In particular, protozoal OM in the rumen could be either increased or decreased with increases in fluid passage rates, the amount depending on the basal passage rate and dietary characteristics (Chapter 4), thus affecting turnover and efficiency in the rumen. An elevated intake level rapidly increased efficiency with the high roughage diets, but less so with the high concentrate diets, whereas a decrease in efficiency was predicted for the all concentrate diet (Figure 1D). The effects would have been even more pronounced if the concentrate had contained lower amounts of fibrous-rich by-products, instead of starch or sugar rich ingredients. Thus, the predictions suggest that the effect of intake level on microbial efficiency depends on the roughage:concentrate ratio, and *vice versa*. A number of experiments have been performed to determine these relationships and results reported have been conflicting. Robinson & Tamminga (1984) obtained a curvilinear response of efficiency to the OM intake per unit body weight. Sniffen & Robinson (1987), and Clark *et al.* (1992), stated that several studies had not indicated a relationship between intake and microbial yield, but with some diets, especially those fed to dairy cattle, a positive relationship had been obtained (Sniffen & Robinson, 1987). In the review of Demeyer & Van Nevel (1986), it was concluded that mixed diets, and non-silage all roughage diets, resulted in higher efficiency figures than concentrate or silage based diets, but that the level of feeding and protein supplementation could alter this ranking. Tamminga (1982b) found no relationship between the roughage:concentrate ratio and efficiency, while elsewhere it was suggested that efficiency was greatest with diets containing 70% roughage (Robinson & Tamminga, 1984) or with diets containing 30-70% roughage (Hagemeister *et al.* 1980). The results in Figure 1D support such optimal roughage:concentrate ratios at intake levels ranging roughly between 11 and 14 kg DM/d for dairy cattle (i.e. between 1.9 and 2.4 % of body weight), but differences in this range were small in predicted efficiency.

To summarize: by consideration of the mechanisms involved, the predictions indicate the variability in efficiency figures as affected by characteristics such as intake level, roughage:concentrate ratio, and protein availability. This integration

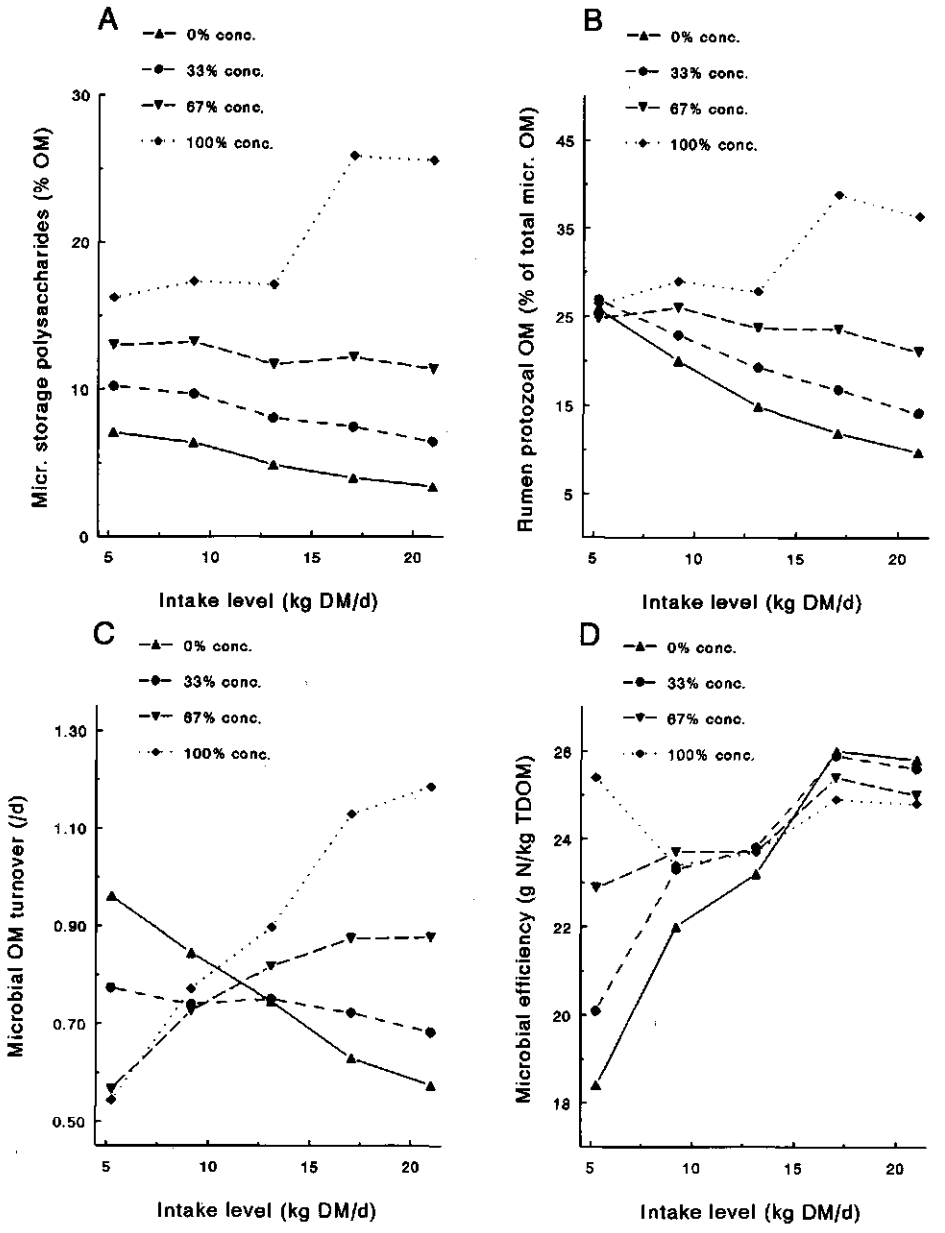

Figure 1. Simulated effects of intake level (kg DM/d) and concentrate content (%) of the diet on the microbial storage polysaccharides (% of OM; graph A), proportion of protozoa in total microbial biomass (graph B), microbial OM turnover (/d; graph C) and microbial efficiency (g N/kg TDOM; graph D).

offers a means of evaluating and explaining the responses to these factors which have been observed experimentally. In contrast, current protein evaluation systems, though including some of the main mechanisms occurring in the rumen, apply fixed efficiency constants. While the use of these empirical systems to predict the amount of protein reaching the duodenum is probably unavoidable in the near future, the prospects of the mechanistic approach adopted in the present study are considered good.

Prediction of VFA in the rumen

VFA production is of considerable importance to the ruminant. Firstly, VFA make a large contribution to the total amount of digestible energy in ruminants (Sutton, 1985). Small differences exist between the major VFA (acetic, propionic and butyric acid) in ATP yielding capacity per unit digestible energy (Swenson & Dukes, 1984). Secondly, they are precursors of body and milk constituents. All VFA are precursors of long chain fatty acids. The role of propionic acid as a glucose precursor is of particular importance, since there appears to be no net glucose absorption across the PDV from dietary sources in dairy cattle and steers (Huntington, 1990). Propionic acid made a major contribution to hepatic gluconeogenesis in non-fasting cattle (between 46 and 73%; review Huntington, 1990). Thirdly, the VFA affect the hormonal balance in the animal, and could therefore have a major impact on the amount and composition of the milk and meat produced. Thus, intravenously administered propionic and butyric acid, though not acetic acid, stimulated insulin secretion to a greater extent than glucose did (Bergman, 1990). However, the physiological importance of this is uncertain, because these acids are metabolized in gut wall and liver to a varying extent. Differences in the energetic efficiency of utilization of the major VFA are small in beef cattle. Some effects on body composition were found when large amounts of acetic acid were added (review Ørskov & MacLeod, 1990). In dairy cattle, pronounced differences between the individual VFA have been reported concerning their effects on milk production and composition. Thus, acetic acid, which does not stimulate insulin secretion and is a fatty acid precursor, increased milk yield and fat content following infusion in the rumen (as summarized by Thomas & Martin, 1988). The effect of propionic acid was a decrease in the milk yield and fat content and an increase in protein content, possibly related to a rise in the insulin secretion and an increased supply of AA resulting from an elevated liver propionic acid metabolism. Interestingly, increasing the supply of glucose in the small intestine raised milk yield, but slightly reduced the protein content. Finally, butyric acid decreased milk yield even more than did propionic acid, and increased fat content. The effects on milk production of diets, which stimulate high propionic and low acetic and butyric acid concentrations in the rumen, show similar effects to the observations of VFA infusions. These diets reduced milk fat secretion and were often associated with a repartitioning of nutrient use within the body of dairy cattle (Sutton, 1985). Overall, it is apparent that the consideration of individual VFA is

important. In the present energy evaluation systems, attempts to recognize these aspects are made by adjustments of ME to NE through the metabolizability factor (q), but its applicability has been questioned (see first part Discussion). In the present study, evaluation of the model indicated correct predictions of total VFA concentrations in rumen fluid, though VFA production was predicted less accurately (Chapter 2). The latter could be related to problems in estimating VFA production, which have large errors of measurement (e.g. Sutton, 1985). Also, it has been shown that the widely used method of Weller *et al.* (1967) does not give particularly accurate approximations of VFA production rates, the results being dependent on the choice of infusate (France *et al.* 1987a). Moreover, most methods assume that the production rate of individual VFA is proportionally the same as their concentrations in rumen fluid. In Chapter 3 however, it has been shown that marked differences occurred in the absorption rates due to pH and individual VFA concentration, which would invalidate this assumption. These considerations suggest that errors in prediction of VFA production could be the result of incorrect assumptions or parameter values in the model, as well as of incorrect measurements. Molar proportions of individual VFA were not predicted well (Chapter 2). Although a relatively large part of the error was attributable to the overall bias of the predictions, indicating that a portion of the error could be eliminated by a mean correction factor, deviation of the regression slope from 1.0 made also a large contribution. The latter indicates a proportional bias due to the inadequate representation of the relationships involved. Because carbohydrate and total NAN flows were predicted well for a range of diets, these results suggest that the stoichiometric coefficients derived by Murphy *et al.* (1982) from a statistical analysis of a large data set are not applicable to the diets used for evaluation in combination with the concepts of the present models. VFA molar proportions as predicted by Beever *et al.* (1981a), Baldwin *et al.* (1987b) and the updated version of the latter model (Argyle & Baldwin, 1988) did not respond accurately to dietary characteristics either (see Chapter 2 for full discussion). In order to predict VFA molar proportions accurately, other coefficients are needed which take into account the dynamics of VFA production and absorption in the rumen.

Generally, the need to maintain redox balance through reduction and reoxidation of pyridine nucleotides (NAD) control fermentation reactions and this affects the energy yield obtained and fermentation products formed (Macfarlane & Gibson, 1993). The methanogenic bacteria are of specific interest, in that they maintain a very low partial pressure of hydrogen, favouring a maximal flow of carbon towards highly oxidized VFA endproducts which have been produced by other species (Wolin & Miller, 1988). A number of factors are involved in the maintenance of redox balance and consequently affect the pattern of VFA in the rumen. These include the composition, amount and rate of depolymerization of the substrate available, and substrate preferences and fermentation strategies of individual species present. The highly integrated nature of the fermentation processes prevents conclusions with respect to a single factor. Thus, while fermentation of a diet with a high woodpulp

(NDF content 99% of DM), high starch or high glucose content resulted in molar proportions of acetic, propionic and butyric acid of 74:18:5, 60:25:10 and 38:22:26, respectively (Ørskov & Oltjen, 1967), these patterns were probably also affected by differences in pH (6.9, 6.7 and 5.7, respectively) and microbial species present. High substrate concentrations, resulting in high growth rates, generally cause a shift in fermentation pattern from acetic acid to butyric and lactic acid *in vitro*, because these latter two act as a sink which disposes of excess reducing power (Cummings & Macfarlane, 1991). In contrast, during carbon limited growth, acetic acid formation is optimal. Russell (1984) showed that increasing *in vitro* growth of *Streptococcus bovis*, decreased acetic acid and increased lactic acid proportions. During starvation, *Selenomonas ruminantium* produced acetic acid only, and during growth produced both acetic and propionic acid (Mink & Hespell, 1981). Thus, differences in the rate of depolymerization of the same chemical component, resulting in differences in the rate of growth, could lead to differences in the molar proportions of VFA formed. It is impossible to confirm such relationships *in vivo*, because of confounding with the substrates degraded and differences in pH. Experiments in which passage rates were increased, and therefore also had increased microbial growth rates, have not shown conclusive results. Harrison *et al.* (1976) reported increased acetic and butyric acids, and decreased propionic acid molar proportions with a purified diet which had an increased fluid passage rate, but rumen α-linked glucose degradation also decreased as well as cellulose degradation increasing. On 90% and 40% roughage diets, increases in fluid passage rate were accompanied by increases in the molar proportions of acetic acid, decreases in propionic acid and either increases or decreases in butyric acid (Harrison *et al.* 1975). Kennedy *et al.* (1976) and Kennedy & Milligan (1978) exposed sheep, fed on brome grass diets, to cold, and observed increased fluid and solid passage rates as well as increased propionate and decreased butyrate proportions in rumen fluid. Bacteria have been observed to shift pathways in response to changes in pH. Decreasing the pH *in vitro* caused acetic molar proportion to decrease and propionic and butyric molar proportions to increase during starch fermentation (Marounek *et al.* 1985), but Strobel & Russell (1986) reported an increase in butyric and lactic acid and a decrease in propionic acid molar proportion from starch fermentation when pH was decreased from 6.7 to 5.8 with mixed bacteria *in vitro*. Other substrates (sucrose, cellobiose, xylan, pectin) in the same experiment, however, increased propionic, butyric and lactic acid molar proportions, indicating the presence of pH x substrate interactions. Many microbial species in the rumen are selective fermenters. Hence, differences between species in fermentation pattern reflect to a certain extent, differences in VFA pattern of the preferred substrate degraded and again stress the integrated nature of the processes. Protozoa may be of specific interest in this respect. The main VFA endproducts of protozoa are acetic and butyric acids, while only trace amounts of propionic acids are produced (Russell & Hespell, 1981; Williams & Coleman, 1988). A specific association between protozoa and methanogenic bacteria has been observed (Krumholz *et al.* 1983;

Stumm & Zwart, 1986), enabling a rapid hydrogen transfer and allowing more acetic acid to be formed by protozoa. Removal of protozoa from the rumen did not have a consistent effect on changes of VFA proportions, probably because other effects resulting from defaunation, such as changes in OM degradation, N availability, and pH, obscure the direct protozoal VFA formation effect. Still, a number of authors concluded that defaunation increased the molar proportion of propionic acid at the expense of butyric acid *in vivo*, and *in vitro* studies have confirmed these results (see review Jouany *et al.* 1988). Thus, starch and sugars fermented by bacteria would be likely to yield much more propionic acid, and less acetic and butyric acid per unit substrate fermented, than would fermentation of starch and sugars by protozoa. There are no data available concerning the relative amounts of VFA produced by bacteria and by protozoa in the rumen. Presumably, diets which promote a high protozoal biomass, will result in a major contribution of the protozoa to rumen VFA formation as well. Estimates of protozoal contribution to total rumen VFA formation on the range of diets used previously (to derive the results depicted in Figure 1) varied between 16 and 37%, the pattern closely following the pattern of proportion of protozoa in the total rumen biomass. With respect to the specific pattern of VFA formed by protozoa, such differences in relative VFA contribution could influence VFA molar proportions and it may be worthwhile to take these contributions into account. At present, the complex interactions between protozoa and other factors which affect the profile of VFA produced in the rumen, do not allow the establishment of the definite influence of protozoa on VFA molar proportions found in the rumen. In summary, the prediction of VFA molar proportions was not improved by comparison with existing rumen models. Validation with respect to other rumen fermentation characteristics, however, indicated the likely source of error, viz. the stoichiometric coefficients applied, since these coefficients do not sufficiently account for the dynamic, integrated character of VFA production and absorption processes.

IMPLICATIONS OF THE RESULTS OF PRESENT MODELS TO FUTURE FEED EVALUATION

In the previous section, the contribution of models to the integration of fermentation processes and prediction of the nutrient supply was discussed in relation to the general concepts and assumptions described in the first part of the Discussion. In several aspects, the models represent a substantial change to previous models. In particular, the role of several microbial groups, including protozoa, in efficiency of microbial synthesis and turnover within the rumen have been depicted. Although predictions of nutrient supply could not always be fully validated, the results suggested that outflow of NDF, starch, sugars and total N were predicted well, that the prospects to predict duodenal AA flow were satisfactory, but that the type of VFA formed was not predicted adequately. Baldwin *et al.* (1987a) indicated that fermentation processes in the colon could be represented relatively simply. Therefore,

given the major objective of the models and the effects of type of VFA on product and product composition, further consideration of the modelling and prediction of VFA production in the rumen should have a high priority. Results of this study could form the basis for such further research. In Chapter 3, VFA absorption rates as affected by concentration, pH and liquid volume have been quantified. The representation of amylolytic and cellulolytic bacteria and protozoa could be used to represent differences in VFA production due to the particular microbial species which ferment the substrate. Improved accuracy of prediction of individual VFA production is then likely to result from application of parameters, providing relationships between substrate fermentation and VFA produced, accounting for the type of substrate, its rate of hydrolysis, and pH of rumen fluid. The classification into five substrates (cellulose, hemicellulose, starch, soluble sugars, and protein) and into two diet types (forages and concentrates) applied by Murphy *et al.* (1982) to derive VFA stoichiometric parameters, give some consideration to the pH and substrate hydrolysis rate, but is likely to be an oversimplification of the true relationships. In this respect, it is important to consider the recycling of OM within the rumen. Some of the VFA are produced from recycled OM and the amount and type of this OM could affect the VFA molar proportions. Again, the models developed, offer opportunities to estimate the type and amount of OM recycled within the rumen.

Major drawbacks identified in the models developed, include the necessity of defining parameters such as the pH and fractional passage rates. The application of appropriate information to establish these driving variables will result in satisfactorily predicted profiles of nutrient supply, as discussed in the previous section. Thus, in the case of predictive models, there is no urgent need to improve this representation. However, in research models there is indeed a need to integrate the mechanisms of passage of fluid and particles and of microbial degradation, in order to achieve progress in the understanding and quantification of the rate of passage (Faichney *et al.* 1989). Moreover, a mechanistic approach to the dynamics of particles (degradation, comminution, passage) in the rumen, and of the metabolic processes within the animal, could in the long term provide more accurate predictions of feed intake (Gill & Beever, 1988; AFRC, 1991). Prediction of intake is of clear significance to feed evaluation, if nutrient supply is to be calculated in animals fed *ad lib*. As discussed previously, current feed intake predictions are applicable for only a limited range of diets and animal and environmental situations. Mechanistic approaches to voluntary feed intake are concerned with the different mechanisms which control feed intake and their interactions, providing a possible integration of the numerous factors affecting voluntary feed intake. The models in the present paper could contribute to such an approach by predicting the metabolic activities of the microorganisms and the resulting fermentation products, and by providing estimates of nutrient availability, to be used in models of metabolism in the body. Attempts to include rumen models into models which predict feed intake, have been described. Thus, Illius & Gordon (1991) developed a model to predict intake and digestion in ruminants, and included

the approach to modelling fibre kinetics described by Mertens (1977). Hyer *et al.* (1991) used the rumen model of France *et al.* (1982) to predict voluntary intake in grazing cattle and concluded that the inclusion of the fermentation component of this rumen model responded well in predicting the various nutrient contributions to rumen fill. The results of the VFA absorption experiment described in Chapter 3 are important in this respect as well. In this Chapter, it was shown that high concentrations of propionic and acetic acid, tended to decrease the fractional absorption rate, and that high rumen liquid volumes reduced absorption rates as well. This would lead to an increase in the proportion of VFA removed by passage to the omasum. In Chapter 3, it was calculated that in dairy cattle, the estimated contribution of passage to total VFA disappearance increased from 20 to 35% when DMI was increased from 5.3 to 21.0 kg/d. In some instances, voluntary feed intake may be controlled by the rate of VFA absorption from the rumen or the ratio of VFA absorbed to other nutrients absorbed (Tamminga & Van Vuuren, 1988; Ketelaars & Tolkamp, 1991). As shown previously in this Discussion, the models described in Chapter 1 and 4 apply in the steady state situation, and so do not predict patterns of VFA concentration in rumen fluid nor rumen pool sizes of degradable and undegradable fibre, even though both may act as feedback signals and therefore may be used in predicting voluntary feed intake (AFRC, 1991). The first detailed representation of particle size and particle size reduction integrated with degradation has been provided by Baldwin *et al.* (1987b) and offers a promising way of simulating diurnal patterns of substrates and endproducts in the rumen. With respect to the impact on production and utilization of nutrients, the effect of discontinuous feeding regimes on patterns of pH and metabolite concentrations in the rumen have been frequently described (Robinson, 1989). Much less is known about the pattern of absorption of nutrients from the digestive tract. Reynolds & Huntington (1988a, b) reported differences in the appearance of nutrients in the portal vein of steers related to the frequency of feeding of lucerne diets (every 2h or twice daily). However, measurements were only made over a limited period during the feeding cycle, which may have enhanced these differences. In case of differences in the appearance of nutrients in the portal vein due to feeding frequency, the utilization and efficiency of utilization of nutrients in the liver may be changed, but there are few data available to test this suggestion. Despite the pronounced effects of feeding frequency on rumen metabolite concentrations and pH, and metabolite appearance in the portal vein, there is little experimental evidence of increased milk yield, although milk fat content has been shown to be affected by feeding frequency (review Robinson, 1989). This may be the result of altered acetate:propionate ratios. However, even the steady state models developed could accommodate such dynamic differences by specifying pH values which depend on the frequency of feeding, after modifications have been made to include the effect of pH on VFA molar proportions. Rumen simulation models, which take account of the feeding frequency, could also offer a possible means to quantify the synchronization of the available carbohydrate and N

sources. Rumen fibre degradation has been shown to improve when N sources were provided more frequently, but results vary considerably (Dixon, 1987). Besides, the efficiency of microbial synthesis could be expected to increase, thus leading to reduced losses of N (through absorption of ammonia) to the environment (Tamminga, 1992).

At present, a general problem with mechanistic approaches is an ignorance of individual variation in performance between animals. Thus, predictions of models give an average value of a group of animals fed the same diet in the same environment, whereas individual variations might exist (Curnow, 1986). For example, Ørskov *et al.* (1988) found consistent differences in outflow rates between cows with *ad lib* or restricted intakes. This makes correct predictions for each individual extremely difficult, because it requires knowledge of the underlying individual factors responsible for these variations. Besides, genetic capacities need to be accounted for. Oldenbroek (1986) made comparisons between breeds fed all-roughage or 50% roughage diets and found a breed x diet interaction for feed intake, milk and protein yield and fat percentage, which indicates differences in the capacities of animals to ingest and utilize feed, dependent on dietary characteristics. To summarize: the explicit representation of discontinuous feeding patterns is probably of minor importance in predicting the profile of available nutrients, except for the differences in VFA molar proportions due to feeding frequency. However, it is of considerable value in the integration of metabolic processes in order to elucidate the mechanisms influencing passage of material out the rumen and voluntary feed intake.

Having established the need to predict VFA patterns more accurately and, for research purposes, the need to represent discontinuous feeding patterns and particle dynamics, the next step is to develop models which predict responses to given inputs. As discussed in the first section, current rationing systems are usually based on separate empirical models of energy and protein supply and utilization, which aim to meet requirements for given outputs. Empirical energy and protein ruminant response models have been constructed and evaluated (see e.g. Geisler & Neal, 1979; Bruce *et al.* 1984; Broadbent *et al.* 1984; Hulme *et al.* 1986). However, like the current rationing systems, marginal cost analysis is not possible since these models are not suitable for prediction of the product composition in relation to dietary inputs. In order to evaluate carcass composition or milk constituents, linear regression models have been developed and evaluated with variable success (see e.g. Harries *et al.* 1976; Rook, 1991). As discussed previously, longer term progress will result from mechanistic models which simulate the response of animals by representation of the metabolism of nutrients by individual tissues. Current mechanistic models of nutrient utilization in beef cattle (e.g. Oltjen *et al.* 1986; France *et al.* 1987b; Di Marco *et al.* 1989; Buchanan-Smith *et al.* 1990) and dairy cattle (e.g. Baldwin *et al.* 1987a; Danfær, 1990) are not yet suitable for predictive purposes, primarily because of insufficient knowledge of the mechanisms involved. Future prospects for mechanistic predictive models, however, are considered good (MacRae *et al.* 1988;

Gill & Beever, 1988). A major limitation identified in several of these mechanistic models is the lack of adequate data concerning the profile of nutrients absorbed (Gill *et al.* 1984; France *et al.* 1987b; Buchanan-Smith *et al.* 1990). In this respect, the framework of the models developed in the present study could be of considerable value in providing estimates of the nutrient profile, particularly when the type of VFA produced and the AA composition of duodenal protein flow can be predicted accurately. Next, central is the prediction of how these nutrients are used by the ruminant for the competing processes of body cell maintenance and service functions (maintenance), fetus and body protein and fat deposition (pregnancy and growth), and lactose, protein and fat secretion in milk (lactation). In the current metabolism models, the partitioning of nutrients have been principally represented according to substrate concentration, based on Michaelis-Menten kinetics. The use of these mathematical expressions is generally acknowledged in animal modelling (Gill *et al.* 1989; Sainz, 1990). Gill *et al.* (1989) mentioned three useful properties for nutrient partitioning of these Michaelis-Menten equations, viz. that all parameters can have a biological meaning ascribed to them; that priorities between fluxes for a particular nutrient are reflected in affinity constants; and that hormonal influences can be mediated through changes in parameter values. Often, the relationship between plasma concentrations of substrates and substrate uptake by tissues do indeed follow Michaelis-Menten kinetics (including aceto-acetate, non-esterified fatty acid, ß-hydroxybutyric acid, AA) (Baldwin *et al.* 1980; Miller *et al.* 1991). However, uptake of glucose and lactate was not determined by plasma arterial concentration, suggesting that factors in addition to glucose concentration can modify glucose uptake. Probably, accommodation has to be made for the effects of hormones on the maximum rates of utilization and the affinities of tissues for substrates. Whilst the role of hormones in metabolic regulation, and consequently in determination of animal responses, is large (e.g. Freedland & Briggs, 1977; Bauman & Currie, 1980), quantitative expressions of their effects have received only limited attention in current animal metabolism models (Sainz, 1990). Merely the models of sheep (Sainz & Wolff, 1987) and dairy cattle (Baldwin *et al.* 1987a) have provisions for endocrine control of metabolism, represented by functions of glucose concentration. The availability of alternative approaches for the representation of hormonal effects, and the lack of quantitative data on hormonal factors limits such an explicit representation of the roles of hormones. In metabolic models, a simple representation of hormonal effects is obtained by simulating the relative availability of a substrate versus its references state. For example, in situations of simulated glucose deficit, gluconeogenesis and lipogenesis were stimulated, and body fat storage and glucose oxidation deactivated, thus simulating hormonal control of nutrient flux without explicitly stating hormone concentrations (France *et al.* 1987b; Pettigrew *et al.* 1992). However, with the latter model, the same initial parameters could not simulate hormonal effects on a wide range of diets (McNamara *et al.* unpublished results). In view of this inaccuracy and given the likely refinements of current models, inclusion of a representation for

endocrine control clearly merits further study.

Whilst the representation of metabolites by plasma concentrations in metabolism models is attractive for its simplicity, it should be noted that the metabolism of substrates, particularly the PDV and the liver, will significantly affect the profile of substrates available for production tissues. These organs are metabolically very active and account for a significant part of the whole body heat production (Reynolds & Tyrrell, 1989). Thus, the type and amount of nutrients reaching the productive tissues can differ markedly from those absorbed from the gastrointestinal tract. The results of a study showing that even for high concentrate diets, no net absorption of glucose from dietary sources across the PDV occurred (Huntington, 1990), have been mentioned in a previous section, as has the preferential metabolism of butyrate and, to a lesser extent, propionate by the gut wall (Bergman, 1990). Equally, the composition of AA absorbed from the gastrointestinal tract and that found in the peripheral circulation also differed substantially (Reynolds & Huntington, 1988a; Reynolds & Tyrrell, 1989). Besides, endogenous protein secretion (enzymes, mucus, epithelial cells) represents a significant contribution to protein flow in the intestines (Swanson, 1982). This may affect AA profiles, and resynthesis of endogenous protein requires both energy and AA, the latter considerably more than re-incorporated into the protein synthesized (Simon, 1989). In some metabolism models, body and visceral protein and fat pools have been distinguished (e.g. Baldwin *et al.* 1987a; Buchanan-Smith, 1990), while the model of Danfær (1990) included a representation of intestinal wall and liver metabolism. However, data concerning the metabolism in gut and liver tissue are scarce and there is limited knowledge available of the factors governing the utilization of nutrients by gut wall and liver (including dietary factors such as intake level and roughage:concentrate ratio). With respect to the impact of gut wall and liver metabolism on the amount and type of nutrients available for productive tissues, more information is required to represent these processes mathematically. This conclusion is supported by recently reported results of a model of liver metabolism in dairy cattle (Freetly *et al.* 1993).

To summarize this section, the progress made and some future directions for new approaches to feed evaluation have been identified. The need to establish accurate predictions of VFA profiles, as well as the need to represent metabolite transformations in gut wall and liver, as well as hormonal effects, have been emphasised, but for adequate representation, more quantitative information is required.

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SUMMARY

SUMMARY

In ruminants, the profile of nutrients available for absorption generally differ largely from that potentially present in the ingested feed. The type and amount of nutrients absorbed can significantly affect the amount and composition of products (milk, meat, wool). Thus, there is an obvious need to the mechanisms responsible for the transformation of ingested to absorbed nutrients. The response of the profile of available nutrients to variations in nutrients entering the rumen is the result of complex interactions occurring during the fermentation. Whilst research on various aspects of rumen fermentation has yielded valuable information on these fermentation processes, the concentration of research on individual components rather than on its integration has resulted in insufficient information on many important mechanisms which link the individual components, and thereby hampers adequate predictions of the supply of nutrients. Mathematical modelling of the fermentation processes provides a means of achieving this integration and for improvement of our understanding of the mechanisms involved, so as to predict the nutrient profile. Such an integration could in the long term improve level and efficiency of animal production with respect to feed input and desired product and product composition, and reduce the output of waste.

The main aim of the present study is the mathematical modelling of the rumen fermentation processes to obtain the integration and quantification of these processes. Considerable progress in modelling rumen function has been made over the years, particularly due to the formulation of improved concepts as knowledge of the subject increased. However, earlier models did not include certain aspects of rumen function which are now recognized as being of major importance in the overall transformation of ingested to absorbed nutrients. In the present study, models of nutrient digestion, absorption and outflow in the rumen were developed which addressed a number of these previously absent concepts. In Chapter 1, a mathematical model of rumen fermentation is described, and the evaluation of this model is reported in Chapter 2. The objective of this dynamic, mechanistic model was to examine the effects of the diet on the profile of nutrients available for absorption in cattle. The equations constituting the model were described by Michaelis-Menten or mass action forms. Parameter values were calculated from the literature or assigned arbitrarily. Given the specific issues which previous models either did not represent or represented inadequately, the present model represents a substantial change from those published earlier. Considerable emphasis has been placed on the representation of microbial metabolism. Aspects of improvement in the mathematical representation of rumen processes include microbial substrate preference, differential outflow and chemical composition of rumen microbes, recycling of microbial matter within the rumen, uncoupling of fermentation with respect to nitrogen availability, reduced microbial activity at reduced rumen pH, and pH-dependent absorption of

volatile fatty acids and ammonia.

Evaluation of this model was described in Chapter 2. Sensitivity analysis on high fibre, high starch and high protein diets, indicated that the model responded appropriately to these types of diets and to changes in parameter values. The model appeared to be sensitive to the availability of hexose for non-growth microbial processes, and to the maximum storage rate of polysaccharides in amylolytic microbes, although sensitivity varied with the composition of the diet. The model was also particularly sensitive to fluid and solid passage rates and to the fraction of protozoa in the amylolytic microbial pool, all input parameters of the model. Model predictions were compared with values observed for a wide range of dietary inputs (dry matter (DM) intake 5-24 kg/d; 25-100% roughage in diet DM). There was satisfactory prediction of duodenal flows of neutral detergent fibre (NDF), total non-ammonia nitrogen (NAN), and total volatile fatty acid (VFA) concentrations. Further partition of NAN flow into microbial and non-microbial NAN parts showed that these components were predicted less accurately. Equally, there were considerable differences between the observed and predicted molar proportions of VFA in the rumen. The reasons for these discrepancies have been given in Chapter 2. From the results of model development (Chapter 1) and model evaluation (Chapter 2), it was concluded that the representation of the complex interactions between rumen microbial populations, particularly the metabolism of protozoa, and of their effects on the production of specific VFA merits further study.

Some part of the inaccurate prediction of VFA molar proportions (Chapter 2) might be attributed to insufficient representation of individual VFA absorption rates, because quantitative information on the factors governing VFA absorption are rare. Therefore, an experiment was performed to quantify the effects of rumen liquid volume, pH, and concentration of VFA, on the fractional absorption rates of acetic, propionic and butyric acids from the rumen of lactating dairy cows (Chapter 3). Experimental solutions of known VFA concentrations but which were at different volumes, pH and concentrations, were introduced into the emptied, washed rumen. Fractional absorption rates were reduced by increasing the amount of solution introduced. An increase in the initial pH of the solution, decreased the absorption rates of all acids, but the effect was more pronounced when the chain length of the acid was increased. Concentration effects were less pronounced. Acetic acid absorption at low concentrations was lower than at medium concentrations, while the absorption rate of propionic acid tended to decrease as the level of concentration increased. These results indicated that the relative concentrations of VFA in rumen fluid might not represent relative production rates. Thus, attempts to estimate individual VFA production from substrate digestion must take into account the volume, pH and VFA concentration.

Evaluation of the developed model (Chapter 2) also indicated the need to represent in more detail protozoal metabolism and the interactions between protozoa and bacteria. The importance of rumen protozoa in the transformation of ingested to

absorbed nutrients is generally recognized. Yet explicit representation has received only limited attention and mathematical procedures to describe the interactions between protozoa and bacteria have not been satisfactorily developed. Therefore, a model was developed in which emphasis was placed on the mathematical representation of the metabolic activities of rumen protozoa (Chapter 4). The objective of this model was to evaluate concepts and data to provide a quantitative understanding of the protozoal dynamics and of the integration of protozoal functioning with other microorganisms and diet composition. Parameters in this model were often assigned values on a priority basis, given relevant experimental observations. The general behaviour of the model was satisfactorily. Comparisons between experimentally observed and predicted protozoal biomass showed general agreement, but the protozoal turnover time was predicted less accurately. Sensitivity analyses indicated that the model was particularly sensitive to the rate of bacterial engulfment, and protozoal maintenance requirements and fractional death rates. Simulations were performed in which dietary components were varied independently, to assess the effects of chemical entities in the feed. Protozoal biomass rapidly increased in response to increases in dietary starch content, but with high concentrate diets further increases in starch caused protozoal biomass to decline due to a rapidly increased death rate. Equally, moderate elevations of the dietary soluble sugar level, increased protozoal biomass, though protozoal biomass was predicted to decrease with high soluble sugar contents of the diet, particularly with high concentrate diets. These simulation results were qualitatively in agreement with data from the literature. Variations in dietary NDF content did not change the simulated protozoal biomass. Reduced dietary nitrogen content decreased the simulated bacterial growth efficiency and total bacterial biomass, and this resulted generally in an increased protozoal biomass. Protozoal biomass either increased or decreased due to changes in feed intake level, depending on the dietary composition and basal intake level. This integration of microbial and dietary characteristics by mathematical representation provided a quantitative understanding of the mechanisms of response of protozoa to changes in dietary inputs. It was also shown that such changes in the relative amounts of bacterial and protozoal biomass in the rumen affected the profile of nutrients available for absorption.

The model described and evaluated in Chapter 4 was used to examine the contribution of protozoa to NDF degradation (described in Chapter 5). Research into the degradation of fibre in the rumen is of particular interest in view of the increased utilization of roughages by cattle herds in the Netherlands, as well as the widespread use of high-fibrous diets in developing countries. The application of mathematical modelling in order to establish the protozoal contribution to NDF degradation allowed the integration of the mechanisms involved. This approach is of considerable importance, because it is impossible to study the protozoal contribution directly *in vitro* and results of defaunation studies have not been conclusive. Simulations were performed for diets in which the concentrate content was varied between 0 and

100% of diet DM and fed at levels ranging between 5.3 and 21.0 kg DM/d. Generally, the simulated protozoal contribution to NDF degradation decreased with increases in intake level or increases in concentrate levels and amounted to 5-35% of the total rumen NDF degradation. However, cellulolytic bacteria were predicted to disappear on all-concentrate diets fed at levels exceeding 15 kg DM/d, and the very low NDF degradation in the rumen was entirely related to the activities of the few protozoa remaining in the rumen for these diets. When dietary NDF levels were reduced, or starch and sugars levels increased independently, there was an increase in the contribution of protozoa to NDF degradation. The protozoal contribution generally declined in response to added nitrogen as well. Such changes in the predicted protozoal contributions to NDF degradation, provided possible explanations for differences in rumen NDF degradation observed *in vivo* when animals were defaunated.

In the General Discussion, the limitations, contributions and conclusions of the results described in previous chapters are discussed with respect to the integration and quantification of rumen fermentation processes, as well as the implications of these results for feed evaluation systems. The usual approach in current feed evaluation systems is to establish the requirements for nutrients to sustain a certain level of production. In the first part of the Discussion however, it is argued that further progress in the field of feed evaluation needs a shift from the current approach towards an approach of predicting responses in product and product composition which result from changes in feed input. Also, it is stressed that it is necessary to recognize metabolism of individual substrates within the rumen or available after absorption from the gut. Finally, it was argued that the models provide an integration and quantification of the processes involved in transformation of ingested to absorbed nutrients. For research type of models, the major limitations identified in the developed models include the necessity of defining pH and fractional passage rates before simulations are performed. Emphasis was placed on the need to integrate the mechanisms of fluid and particle passage and of microbial degradation in order to achieve progress in perception and prediction of passage of material out of the rumen and in voluntary feed intake. Besides, the detailed representation of protozoal and bacterial metabolism allows evaluation of the effects of diet on the turnover and efficiency of synthesis of microbial matter in the rumen. For predictive purposes, the framework of the models developed is of considerable value in providing estimates of the nutrient profile available for absorption. However, given the impact of the type of VFA on product and product composition, and the inaccurate predictions from models of rumen fermentation, further consideration of the prediction of VFA production in the rumen is identified as an area of high priority. The results of both the *in vivo* experiment and the models developed in the present study, could form a suitable basis for further consideration of VFA prediction. Subsequently, an improvement of predictions of the responses of ruminants will be achieved through a recognized interaction of individual nutrients available for absorption at organ and tissue level.

Main conclusions

The mathematical models developed in the present study have addressed a number of aspects of rumen function which were not included in previous models, but have been recognized as being of major importance in the transformation of ingested to absorbed nutrients. In particular, representation of microbial metabolism has been improved and as such the models developed represent a substantial change to previous models.

Results of model evaluation suggested that the outflow of nutrients from the rumen (NDF, starch, soluble sugars, nitrogen) were predicted well on a wide range of dietary inputs. However, the type of VFA formed was not predicted satisfactorily and possible reasons for this inaccurate prediction have been identified.

The absorption of the main VFA in the rumen is affected to a variable extent by volume, pH and VFA concentration. Hence, molar proportions of VFA in rumen fluid do not necessarily represent the proportions in which they are formed. These factors should be taken into account if production of individual VFA in the rumen is to be predicted accurately.

Major aspects of rumen protozoal metabolism have been represented mathematically. The mathematical integration of protozoal, bacterial and dietary characteristics provided a quantitative understanding of the mechanisms of protozoal responses, and their effects on nutrients available for absorption, to changes in dietary inputs.

The prediction of response to dietary inputs of the amount and composition of the product should recognize the metabolism of individual substrates within the rumen or available after absorption. The models developed provide a basis for the estimation of the profile of nutrients available for absorption and thus, ultimately for production. With respect to the prediction of these responses, further consideration of the prediction of type of the VFA formed in the rumen should have a high priority.

SAMENVATTING

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Bij herkauwers is er een aanzienlijk verschil tussen hoeveelheid en aard van nutriënten die met het voer worden aangeboden en die welke voor absorptie beschikbaar zijn. Het profiel van voor absorptie beschikbare nutriënten kan een wezenlijke invloed hebben op de produktie van de herkauwer, zowel in termen van absolute hoeveelheden produkt (melk, vlees, wol) als de samenstelling van het produkt. Het belang van het onderzoek naar mechanismen die aan deze drastische verandering van nutriëntenprofiel een bijdrage leveren is daarmee duidelijk. De door veranderingen in voeropname en voersamenstelling veroorzaakte effekten op het profiel van nutriënten die uit het maagdarmkanaal geabsorbeerd kunnen worden, komen tot stand via veranderingen in de fermentatieprocessen in de pens. Onderzoek naar de verschillende facetten van pensfermentatie heeft waardevolle informatie opgeleverd over deelaspekten ervan. Het onderzoek naar de integratie van die deelaspekten heeft echter aanzienlijk minder aandacht gekregen en dit belemmert een juiste voorspelling van het aanbod van voor absorptie beschikbare nutriënten. Als goed bekend is hoe het penssysteem werkt en op welke wijze effekten tot uiting komen (kennis), dan zijn schattingen te maken en kan er sturing plaatsvinden (voorspelling). Een belangrijke bijdrage aan de integratie van deelaspekten van pensfermentatie en de toename van kennis over de verschillende mechanismen die hierbij betrokken zijn, kan geleverd worden door het wiskundig modelleren van de processen in de pens. Op langere termijn kan deze integratie de efficiëntie van produktie verbeteren, met name met betrekking tot de gewenste samenstelling van het produkt. Bovendien zou de uitstoot van milieubelastende componenten (mest, urine, gassen) verminderd kunnen worden. Het grootste deel van het in dit proefschrift beschreven onderzoek is gericht op de wiskundige modellering van pensfermentatieprocessen, met het doel deze processen te integreren en te kwantificeren.

Gedurende de laatste twee decennia is aanzienlijke vooruitgang bereikt in het wiskundig weergeven van de fermentatieprocessen in de pens, met name doordat de kennis over deelaspekten ervan telkens toenam en wiskundige principes verder werden ontwikkeld. Toch waren reeds ontwikkelde modellen onvolledig in de weergave van een aantal aspekten, die nu algemeen als van bijzonder belang in het proces van omzettingen worden beschouwd. In het onderzoek beschreven in dit proefschrift werden modellen van de fermentatie in de pens ontworpen waarin deze tot nu toe onvolledig weergegeven facetten wel werden opgenomen. In Hoofdstuk 1 is een wiskundig model van de pensfermentatieprocessen beschreven. Resultaten van simulaties met dit model en model evaluatie worden in Hoofdstuk 2 beschreven. Het doel van dit dynamische, mechanistische model was om, op basis van uit de literatuur bekende processen in de pens, te onderzoeken hoe rantsoeneigenschappen invloed uitoefenen op de hoeveelheid en aard van nutriënten die uit de pens stromen of uit de pens geabsorbeerd worden. Reaktiesnelheden werden wiskundig beschreven

met behulp van standaard vergelijkingen afgeleid van de enzymkinetiek (Michaelis-Menten vergelijkingen) of, in enkele gevallen, met zogenaamde 'mass action' vergelijkingen. Waarden van parameters in het model werden berekend uit literatuur gegevens. Wanneer geen bruikbare gegevens voorhanden waren, werden aannames gedaan over parameterwaarden. Het model verschilt wezenlijk van eerder ontwikkelde modellen met betrekking tot de specifieke deelaspekten en (vooral) kombinaties van deelaspekten die eerder niet of onvolledig weergegeven waren. Er werd vooral veel aandacht gegeven aan de aktiviteiten van de micro-organismen in de pens. Verbeterd werden met name de wiskundige weergave van de voereffekten op samenstelling van micro-organismen; substraatvoorkeur van micro-organismen; verschillende uitstroomsnelheden van micro-organismen; recycling van micro-organismen in de pens; niet met microbiële groei gepaard gaande afbraak van koolhydraten gerelateerd aan stikstof beschikbaarheid; verminderde microbiële aktiviteit bij lage pH waarden; pH afhankelijke absorptie van vluchtige vetzuren en ammoniak.

De evaluatie van het model bestond uit een gevoeligheidsanalyse en validatie bij een range van qua samenstelling sterk verschillende rantsoenen (Hoofdstuk 2). De gevoeligheidsanalyse werd uitgevoerd met op silage gebaseerde rantsoenen waarin door supplementatie respektievelijk het aandeel NDF, zetmeel of stikstof hoog was. Deze simulaties gaven aan dat het model goed reageerde op deze verschillende rantsoenen. De resultaten bleken afhankelijk te zijn van vooral de beschikbaarheid van hexose voor micro-organismen voor andere dan aan groei gekoppelde doelen en voor de waarde van de parameter die de maximale snelheid van vorming van reserve koolhydraten weergeeft. Overigens was de gevoeligheid voor parameters afhankelijk van het type rantsoen. Daarnaast bleek het model gevoelig voor de input variabelen die de fraktionele passagesnelheid van vloeistof en deeltjes uit de pens definiëren en voor de fraktie protozoën in de pool van amylolytische microben. Model validatie gebeurde door experimenteel gevonden waarden bij verschillende rantsoenen (droge stof opname 5-24 kg/dag en ruwvoeraandeel in het rantsoen variërend tussen 25 en 100%) te vergelijken met waarden voorspeld door het model. De uitstroom van neutral detergent fibre (NDF) en totaal niet-ammoniak stikstof (NAN) naar de darmen en de concentratie van vluchtige vetzuren (VVZ) in de pensvloeistof werden goed voorspeld. De komponenten waaruit de NAN uitstroom bestaat, namelijk die van microbiële en niet-microbiële oorsprong, werden echter minder goed voorspeld. Bovendien waren er grote verschillen tussen experimenteel waargenomen en voorspelde verhoudingen van VVZ in de pens. Een aantal mogelijke redenen voor dergelijke verschillen werden gegeven. Gebaseerd op de resultaten van de ontwikkeling van het model (Hoofdstuk 1) en de evaluatie van het model (Hoofdstuk 2) werd de conclusie getrokken dat zowel de weergave van de complexe interakties tussen micro-organismen, en met name de weergave van de aktiviteiten van protozoën in de pens, als de effekten daarvan op de produktie van VVZ nader onderzoek verdient.

Kwantitatieve gegevens over de faktoren die de snelheid van absorptie van VVZ

uit de pens bepalen zijn schaars. De onjuiste voorspellingen van VVZ verhoudingen in de pens zou deels aan dit ontbreken van gegevens te wijten kunnen zijn. Daarom werd een experiment opgezet en uitgevoerd met als doel de effekten van pH, VVZ concentratie en vloeistofvolume in de pens op de absorptie van azijnzuur, propionzuur en boterzuur uit de pens van melkvee te kwantificeren (Hoofdstuk 3). Vloeistof met bekende concentraties van VVZ werden in de leeggemaakte en gewassen pens gebracht en concentraties van VVZ werden aansluitend iedere 10 minuten gedurende 1 uur bepaald. Een toename in het vloeistofvolume had een daling van de fraktionele absorptiesnelheid van de VVZ tot gevolg. Een toename van de pH van de vloeistof verlaagde de fraktionele absorptiesnelheden van de VVZ. Het effekt hiervan was groter naarmate de ketenlengte van het vetzuur toenam. De fraktionele absorptiesnelheid van azijnzuur was bij geringe concentraties lager dan bij gemiddelde concentraties, terwijl er een tendens tot afname van absorptiesnelheid van propionzuur met een toename van de concentratie werd gevonden. Deze resultaten gaven aan dat de verhoudingen van VVZ in de pensvloeistof niet noodzakelijkerwijs de verhoudingen waarin ze geproduceerd zijn weergeven. Wanneer de produktie van individuele VVZ uit fermentatie van substraat geschat wordt dient er daarom rekening te worden gehouden met de effekten van pH, concentratie en vloeistofvolume.

Evaluatie van het ontwikkelde model (Hoofdstuk 2) maakte bovendien duidelijk dat het metabolisme van de protozoën en de interakties met bacteriën beter weergegeven zouden moeten worden. In de literatuur wordt het belang van de protozoën in de omzetting van opgenomen nutriënten in geabsorbeerde nutriënten algemeen erkend. Omdat protozoën zich moeilijk laten kweken heeft de expliciete weergave van de aktiviteiten van de protozoën maar weinig aandacht gekregen en wiskundige technieken om de interakties tussen protozoën en bacteriën te beschrijven zijn matig ontwikkeld. Daarom werd een model ontworpen (beschreven in Hoofdstuk 4) waarin de nadruk werd gelegd op de wiskundige weergave van de aktiviteiten van de protozoën. Het doel van dit model was om concepten en gegevens van protozoën te evalueren, om zo kwantitatief meer kennis te verkrijgen omtrent de dynamiek van protozoën in de pens en een integratie te bewerkstelligen tussen zowel de aktiviteiten van de protozoën in de pens als het metabolisme van bacteriën en rantsoeneigenschappen. De parameter waarden werden vaak op prioriteitsbasis afgeleid van relevante (meest kwalitatieve) experimentele gegevens. Het gedrag van het model was in het algemeen bevredigend. Waargenomen en voorspelde hoeveelheden protozoën in de pens kwamen goed overeen, maar de voorspelling van de turnover snelheid van protozoën in de pens was minder goed. De gevoeligheidsanalyse gaf aan dat het model gevoelig was voor met name de snelheid waarmee bacteriën door protozoën werden opgenomen, de onderhoudsbehoefte van protozoën en de fraktionele snelheid van afsterven van protozoën. De effekten van afzonderlijke voederbestanddelen op de aktiviteiten van protozoën werden bepaald in simulaties waarin de rantsoencomponenten (NDF, zetmeel, oplosbare suikers en stikstof) onafhankelijk van elkaar werden gevarieerd. Een toename van het zetmeelgehalte van het

rantsoen vergrootte de biomassa van protozoën in de pens. Maar op rantsoenen met een hoog aandeel krachtvoer, en daarmee veel zetmeel en suikers, nam de fraktionele sterfsnelheid dusdanig snel toe met verdere verhogingen van het zetmeelgehalte dat juist een verlaging van de hoeveelheid protozoën voorspeld werd. Gesimuleerde hoeveelheden protozoën veranderden op dezelfde wijze in respons op verhogingen van het oplosbaar suikergehalte van het rantsoen. Dergelijke resultaten komen kwalitatief overeen met veranderingen zoals vermeld in de literatuur. De voorspelde hoeveelheden protozoën veranderden niet wanneer het NDF gehalte van het rantsoen werd gewijzigd. Een verlaging van het stikstofgehalte van het rantsoen leidde tot een verminderde efficiëntie van bacteriële groei en verminderde hoeveelheden bacteriën in de pens, en daarmee in het algemeen tevens tot verhoogde hoeveelheden protozoën in de pens. Afhankelijk van het basis nivo van voeropname en van de rantsoensamenstelling werd een verhoogde of verlaagde biomassa van protozoën voorspeld wanneer het voeropnamenivo toenam. De geïntegreerde wiskundige weergave van protozoën, bacteriën en rantsoeneigenschappen zorgde voor een verbeterd kwantitatief begrip van responsmechanismen van protozoën in relatie tot het rantsoen. Bovendien werd aangetoond dat dergelijke veranderingen in relatieve hoeveelheden van protozoën en bacteriën in de pens het profiel van voor absorptie beschikbare nutriënten beïnvloeden.

Het model, beschreven en geëvalueerd in Hoofdstuk 4, werd toegepast om de bijdrage van protozoën aan de afbraak van NDF in de pens vast te stellen (Hoofdstuk 5). Het onderzoek naar de afbraak van NDF is van speciaal belang gezien het toenemende gebruik van ruwvoer op de Nederlandse rundveehouderij bedrijven en gelet op het belang van ruwvoer voor de rundveestapel in ontwikkelingslanden. Zoals hiervoor al is gemeld, maakt de toepassing van wiskundige modellen om de bijdrage van protozoën aan NDF afbraak vast te stellen een integratie mogelijk van de verschillende faktoren die hierbij een rol spelen. Deze methode van aanpak van het probleem kan van aanzienlijke waarde zijn omdat het niet mogelijk is de bijdrage van protozoën aan NDF afbraak direkt *in vitro* te bestuderen, terwijl de resultaten van onderzoek, waarbij de pens werd gedefauneerd, geen uitsluitsel gaven over deze bijdrage. Simulaties werden uitgevoerd op rantsoenen waarvan het krachtvoeraandeel varieerde tussen 0 en 100% en bij voeropnamenivo's variërend van 5.3 tot 21.0 kg droge stof per dag. In het algemeen nam de gesimuleerde bijdrage van protozoën aan NDF afbraak in de pens af met een toename van het voeropnamenivo of een afname van het krachtvoeraandeel. De bijdrage lag tussen 5 and 35% van de totale NDF afbraak in de pens. Echter, op 100% krachtvoerrantsoenen voorspelde het model het uitsterven van cellulolytische bacteriën bij een voeropname boven 15 kg droge stof per dag. De (zeer lage) NDF afbraak in dergelijke situaties kon volledig worden toegeschreven aan de gesimuleerde kleine hoeveelheid protozoën. Een verhoging van het NDF gehalte of een verlaging van het zetmeel- of suikergehalte, onafhankelijk van elkaar, deed de bijdrage van protozoën aan NDF afbraak toenemen. Deze bijdrage nam echter meestal af wanneer het stikstofgehalte van het rantsoen werd verhoogd.

Dergelijke veranderingen in voorspelde NDF afbraak door aktiviteiten van de protozoën kunnen bijdragen aan het verklaren van verschillen in NDF vertering in de pens die zijn waargenomen *in vivo* bij gedefauneerde dieren.

In de Algemene Discussie is ingegaan op de beperkingen, bijdragen en conclusies van de resultaten beschreven in de Hoofdstukken 1 - 5 in relatie tot het integreren en kwantificeren van de fermentatieprocessen. Bovendien zijn de implikaties ervan voor voederwaarderingssystemen voor herkauwers aangegeven. In het eerste deel van de Discussie is aangevoerd dat verdere vooruitgang op het terrein van voederwaardering een verschuiving vraagt van de berekeningsmethode van behoeften aan nutriënten om een bepaalde produktie te bereiken naar de berekeningsmethode van respons van produktie en produktsamenstelling op veranderingen in het rantsoen. Vervolgens is beklemtoond dat het noodzakelijk is kennis te hebben van het metabolisme van de afzonderlijke substraten in de pens of, na absorptie, in weefsels en organen. Tenslotte is het belang aangegeven van wiskundige modellen in het integreren en kwantificeren van de processen die betrekking hebben op de omzetting van opgenomen in geabsorbeerde nutriënten. Een belangrijke beperking van toepassing van modellen, beschreven in dit proefschrift, voor toekomstige modellen die gericht zijn op het beantwoorden van onderzoeksvragen, is de noodzaak om pH en fraktionele passagesnelheden te moeten definiëren voordat de simulatie plaatsvindt. Dit is van belang, omdat vooruitgang in kennis en voorspelling van passage van materiaal uit de pens en van de maximale voeropname een integratie van passage- en microbiële afbraakmechanismen vereist. Bovendien maakte de gedetailleerde weergave van het microbiële metabolisme een evaluatie mogelijk van de effekten van het rantsoen op recycling en efficiëntie van synthese van microbieel materiaal. Met betrekking tot voorspellingsgerichte modellen kan het kader van de in dit onderzoek ontwikkelde modellen van aanzienlijke waarde zijn voor het schatten van de beschikbaarheid van nutriënten voor absorptie. Echter, gelet op het belang van het type VVZ voor de produktie en produktsamenstelling enerzijds, en de minder goede voorspellingen van de verhoudingen van VVZ anderzijds, dient het onderzoek naar de voorspelling van het patroon van geproduceerde VVZ bij een range van rantsoenen hoge prioriteit te krijgen. De resultaten van zowel het in Hoofdstuk 3 beschreven experiment als de in de andere hoofdstukken beschreven modellen vormen een geschikte basis voor verdere ontwikkeling van modellen voor VVZ voorspellingen. Vervolgens kan de voorspelling van de respons van herkauwers verbeterd worden door de individuele nutriënten, die beschikbaar zijn voor absorptie, en de interakties daartussen op weefsel- en orgaannivo in ogenschouw te nemen.

Belangrijkste conclusies

In de wiskundige modellen die in dit onderzoek werden ontwikkeld zijn een aantal facetten van de fermentatieprocessen in de pens opgenomen, die in reeds eerder ontwikkelde modellen niet of onvolledig waren weergegeven en die nu algemeen als belangrijk voor deze processen worden beschouwd. Met name de wiskundige weergave van het microbiële metabolisme werd verbeterd. In dit opzicht zijn de ontwikkelde modellen wezenlijk verschillend van reeds bestaande modellen.

Resultaten van model evaluatie gaven aan dat de uitstroom van nutriënten naar de darmen (NDF, zetmeel, oplosbare suikers, stikstof) bij een brede range van rantsoenen goed werd voorspeld. De voorspelling van de vorming van het type vluchtige vetzuur was echter minder goed en de meest waarschijnlijke redenen hiervoor werden aangeduid.

De absorptie van vluchtige vetzuren uit de pens werd, afhankelijk van het type vetzuur, beïnvloed door de pH van de pensvloeistof, de concentratie van het vetzuur en het vloeistofvolume in de pens. De verhoudingen van vluchtige vetzuren in de pens hoeven dus niet noodzakelijkerwijs de verhoudingen waarin ze zijn geproduceerd weer te geven. Wanneer de produktie van individuele vetzuren in de pens geschat wordt dient rekening te worden gehouden met dergelijke effekten.

De belangrijkste facetten van het metabolisme van protozoën werden wiskundig weergegeven. De integratie van het metabolisme van protozoën en bacteriën en voedereigenschappen zorgde voor een kwantitatief begrip van de mechanismen betrokken bij de respons van protozoën op wijzigingen in het rantsoen en van de effekten van protozoën op de beschikbaarheid van nutriënten voor absorptie.

De voorspelling van de invloed van veranderingen in het rantsoen op de produktie en produktsamenstelling vereist een kennis over het metabolisme van de afzonderlijke substraten in de pens of beschikbaar na absorptie. De ontwikkelde modellen vormen een basis voor de schatting van de hoeveelheid en het type nutriënten beschikbaar voor absorptie en uiteindelijk voor produktie. Het onderzoek naar de voorspelling van het type vluchtig vetzuur dat in de pens gevormd wordt bij uiteenlopende rantsoenen verdient hoge prioriteit.

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CURRICULUM VITAE

Jan Dijkstra werd op 13 september 1964 geboren in Assen. In 1982 behaalde hij het VWO diploma aan het Gomarus College in Groningen. In datzelfde jaar werd begonnen met de studie Zoötechniek aan de toenmalige Landbouwhogeschool in Wageningen. In juni 1987 studeerde hij met lof af aan de Landbouwuniversiteit Wageningen (LUW) met als hoofdvak Veefokkerij en als bijvak Vruchtbaarheid en Voortplanting. Tijdens zijn studie, vanaf 1 oktober 1986, was hij als assistent-onderzoeker verbonden aan het Instituut voor Veeteeltkundig Onderzoek (IVO) in Zeist. Daar was hij belast met het onderzoek naar de mogelijkheden voor selektie op kalfsvleesproduktie- en stierevleesproduktie-eigenschappen bij rundvee. Vanaf 1 mei 1988 was hij aangesteld als Assistent In Opleiding (AIO) bij de vakgroep Veevoeding van de LUW, waar het onderzoek beschreven in dit proefschrift werd verricht. In 1989 verbleef hij 6 maanden als gastmedewerker aan het AFRC Institute of Grassland and Environmental Research in Hurley (UK). Van 24 augustus 1992 tot 1 mei 1993 was hij aangesteld als toegevoegd onderzoeker bij de vakgroep Veevoeding. In het eerste deel van deze periode werd een verkennende studie met betrekking tot het fundamenteel dierfysiologisch onderzoek (onderdeel stofwisselingsfysiologie) in Nederland voltooid in opdracht van de Nationale Raad voor Landbouwkundig Onderzoek (NRLO). Tijdens het tweede deel van deze periode was hij belast met het coördineren van het onderwijs- en onderzoekprogramma ten behoeve van het Onderzoekinstituut Animal Sciences in opdracht van de cluster Zoötechniek en Zoölogie en de vakgroep Veevoeding.