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Original article

Development of a mechanistic model for rumen digestion validated using the duodenal flux of amino acids

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Summary — A mechanistic model describing rumen digestion is presented. The model consists of 22 compartments for classical nutrients, microbes associated with either solids or fluid, and 8 amino-acid compartments. An empirical approach for volatile fatty acids production is used. Sensitivity and behavioural analysis on several parameters demonstrated the need to improve knowledge concerning certain factors. Model results on general criteria are in good agreement with observed data. Validation on 49 experimental lysine and methionine duodenal fluxes were satisfactory. A comparison between observed and predicted values of LysDi and Metdi on several feedstuffs showed good results. This model is a first step in the building of a rumen model suitable for research and application.

model / rumen / amino acid / validation

Résumé — Mise en place d'un modèle du rumen validé pour les flux duodénaux d'acides aminés. *Un modèle mécaniste de la digestion ruminale est décrit. Il comprend 22 compartiments. Les microorganismes sont séparés en 2 groupes : attachés à la phase particulaire ou libres. Les glucides sont subdivisés en 7 compartiments et les protéines en 5. Quatre compartiments d'acides aminés sont distingués dans chaque phase (lysine, méthionine, acides aminés ramifiés et autres). Les stœchiométries des fermentations ruminales sont déterminées par des relations empiriques issues de la bibliographie. Des analyses de sensibilité portant sur quelques paramètres du rumen montrent la nécessité d'approfondir la connaissance sur certains d'entre eux. Les validations effectuées sur des critères généraux du fonctionnement ruminal sont globalement satisfaisantes. Des validations sur 49 flux duodénaux publiés de lysine et méthionine révèlent une bonne capacité de prédiction de ces flux. Une comparaison entre les valeurs de lysine digestible (LysDi) et de méthionine digestible (MetDi) de l'INRA et du modèle sur quelques matières premières montre une bonne adéquation. Le modèle est une première étape de description du système ruminal pouvant être développé vers la recherche ou l'application.*

modèle / rumen / acide aminé / validation

INTRODUCTION

During the last 30 years many partial or complete models of rumen digestion have been proposed (for review see Sauvant and Ramangasoavina, 1991). The first complete model generally cited is that of Baldwin *et al* (1987) even though other models had already been proposed, namely that of Black *et al* (1980), which were capable of describing the principle phenomena of rumen digestion in a globally satisfactory manner. Danfaer (1990) and Dijkstra (1993) have published the most recent models. However these models can be considered incomplete with regard to present knowledge concerning the rumen, and considering the need to develop equations for rumen digestion in order to assess rations and to define diets in relation to expected responses. For example, the phenomenon of digestive interactions, which is partially integrated in the evaluation of dietary energy values, is not taken into account. In addition, the influence of the level of ingestion on ruminal digestive parameters was considered only by Dijkstra (1993). Moreover, the stoichiometric relationships for the production of volatile fatty acids, used by many authors, are those proposed by Murphy *et al* (1982), while they have often been criticised during model validation and numerous results have since been published. Finally, several research teams (O'Connor *et al,* 1993; Rulquin *et al,* 1993) have already suggested that ruminant feeding should take into account the supply of certain essential amino acids, notably lysine and methionine, while the group of O'Connor also suggested the use of branched-chain amino acids. We propose a model which will contribute to progress by quantitative integration of new information in mechanistic models for the rumen, namely for the prediction of duodenal amino-acid flux and the proportions of the different volatile fatty acids in the rumen.

METHODS

The present model uses the principle of a mechanistic approach to modelise biological systems (France and Thornley, 1984; Sauvant, 1992). The reticulo-rumen is treated as a compartmental system fed by flows into and reduced by flows out of the system. The in-flows are linked to ingestion for the substrates and to the processes of degradation for the microorganisms. The out-flows are linked to the phenomena of degradation and absorption for the substrates and transit for the substrates and microorganisms. The model describes the reticulo-rumen by a number of differential, dynamic and determinist equations. For this purpose, the variation in the quantity of a substance in a compartment during an interval of time is equal to the sum of the related fluxes entering minus the fluxes leaving the compartment. The model therefore contains as many differential equations as there are compartments. The mathematical expressions of flux are established in the simplest possible manner. In particular, we have tried to use linear functions for the quantities of substances in the compartments, to try and avoid the phenomena of instability or chaotic evolution, which can appear in non-linear models (Dahan-Dalmedico *et al,* 1992). With the same aim in mind we have tried to avoid, where possible, negative feedback phenomena which, like latency times, are not essential when one tries to simulate rumen equilibrium over a period of 24 h as in the present case.

The values retained for the different parameters of the model are from experimental results obtained from the literature or in our laboratory. However, in a certain number of cases, the amplitude of the variations in the available values is great and unexplainable. In these situations an estimation was used first in the model which then allowed studies of sensitivity to be per-

formed. In this way the influence of the variations in the least precise parameters on the behaviour of the reticulo-ruminal system could be investigated. In addition to this step of internal validation external tests were performed using information not used in the construction of the model to study the ability of the model to explain experimental phenomena.

The model has been developed with the use of the Dynamo language (Pugh, 1983) and for some aspects ACSL-SimuSolv (MGA, 1991). With Dynamo the method of numerical integration was that of Euler with each time step lasting 1 h and a length of simulation of 6 d. The values retained were those of the 6th day, since it seems to be the optimal time interval necessary for the daily fluxes not to depend on the initial conditions and for the model to achieve dynamic equilibrium. The method of Gear (Steiner *et al,* 1990) was used with ACSL-SimuSolv. Bearing in mind that the model is largely linear and that the compartments are of roughly the same size with homogeneous rates of turnover, the two methods give the same results. The rates are expressed per hour which is an interval of time justified by the fact that ingestion is continuous in this model.

DESCRIPTION OF THE MODEL

The entry parameters

The entry parameters correspond to those for a dairy cow. For each variable the abbreviation and the units will be given. The liveweight (LW, kg) and the amount of milk produced, with 4% fat (MP, kg/d) are used as the base values to estimate empirically the daily quantity of dry matter ingested (DMI, kg/d; INRA, 1988):

$$DMI = 0.27MP + 0.01(LW - 600) + 11 \quad [1]$$

This DMI was used to calculate the usable rumen volume (RV, l; Rémond, 1988).

$$RV = 2.26DMI + 40.5 \quad [2]$$

The ration is defined, at a global level, by the quantities of forage and concentrate ingested where the proportions are defined by F and C (F + C = 1). The chemical compositions of the forage and concentrate are defined from classical chemical analysis (dry matter, organic matter, crude protein or 6.25N and crude fat) and van Soest analysis of cell-wall material (van Soest, 1982). In addition, the level of soluble sugars and starch was also taken into account.

The carbohydrates and proteins in the feedstuffs were broken down into different groups using their chemical characteristics and their degradation profiles estimated by *in sacco* measurements (Orskov and MacDonald, 1979). The crude protein was therefore separated into 3 fractions: soluble and very rapidly degradable (SN); progressively degradable (DN); and undegradable (UN). In addition, the levels of lysine, methionine, branched-chain amino acids and other dietary protein amino acids have been taken into account.

Total carbohydrates correspond to the organic matter minus crude protein and crude fat. It was divided into 4 categories; soluble sugars which corresponds to those measured in cell contents plus the soluble fraction of starch, which can be important (Tamminga *et al,* 1990; Sauvant *et al,* 1994). Non-soluble starch, which is assumed to have a homogeneous rate of degradation. Cell-wall material, estimated by the neutral detergent fiber (NDF) method, is separated into 2 fractions on the basis of *in sacco* measurements, degradable and undegradable. The fats were associated with the minerals and with the aim of simplification their digestion was not studied in the present model.

The rates of digesta passage are calculated from DMI, LW and the proportion of

forage. The empirical relationships used for this calculation have been obtained by Sauvant and Archimède (1989) by adjusting published literature values. This allows the estimation of the rates of forage (K_{pf}) and concentrate (K_{pc}) particle passage and of liquid phase passage (K_l)

$$K_{pf}\ (\%/h) = 0.35 + 22DMI/(LW)^{0.75} + 2F^2 \quad [3]$$

$$n = 152,\ r^2 = 0.80,\ RSD = 0.75$$

$$K_{pc}(\%/h) = 1.25K_{pf} + 0.25 \quad [4]$$

$$n = 36,\ r^2 = 0.86,\ RSD = 0.78$$

$$K_l\ (\%/h) = 2.45 + 25DMI/(LW)^{0.75} + 4F^2 \quad [5]$$

$$n = 138,\ r^2 = 0.79,\ RSD = 2.04$$

Rumen compartments

Protein fractions

The soluble proteins in the rumen (SN) come from the cellular contents of ingested forage and concentrate (IFSN and ICSN, respectively). They can pass into the small intestine with the liquid phase. The soluble proteins are degraded into peptides and amino acids (Chen *et al*, 1987) according to the rate constant K_{sn}, taken from the Cornell system (l/h, Sniffen *et al*, 1992).

$$dSN/dt = DMI(IFSN \bullet F + ICSN \bullet C) - K_l \bullet SN - K_{sn} \bullet SN \quad [6]$$

Two ruminal compartments for progressively degradable proteins are distinguished, one for the forage (FDN) and the other for the concentrate (CDN). The entry fluxes are calculated from the products of the quantities of ingested forage and concentrate multiplied by the respective levels of these proteins (IFDN and ICDN).

The rates of degradation (K_{fdn} and K_{cdn} are the result of *in sacco* measurements published in particular by INRA (Aufrère *et al*, 1992; Michalet-Doreau, 1992) or unpublished results (Chapoutot, personal communication). The degradable proteins pass into the small intestine at the same rate as the solid phase to which they belong:

$$dFDN/dt = DMI \bullet F \bullet IFDN - K_{fdn} \bullet FDN - K_{pf} \bullet FDN \quad [7]$$

$$dCDN/dt = DMI \bullet C \bullet ICDN - K_{cdn} \bullet CDN - K_{pc} \bullet CDN \quad [8]$$

Only one compartment for undegradable proteins (UN) has been considered. The entry fluxes were calculated from *in sacco* measurements of the nitrogen fractions which correspond to the forage (IFUN) and concentrate (ICUN). These proteins flow out of the rumen with the solid fraction at an average rate calculated from the rates K_{pc} and K_{pf} weighted according to the proportions of forage and concentrate in the diet:

$$dUN/dt = DMI(IFUN \bullet F + ICUN \bullet C) - (K_{pc} \bullet C + K_{pf} \bullet F)UN \quad [9]$$

Amino acids (AA) and peptides

The compartment of free amino acids and peptides is in fact made up of degradable proteins which are not fermented since this assumes deamination according to our hypothesis for the construction of the model. The principle of a 2-step ruminal digestion has already been applied in other analytical models of ruminal digestion (van Milgen *et al*, 1991). Four amino-acid compartments have been distinguished in each phase: lysine (LYS), methionine (MET) and branched-chain amino acids (bAA), which

include leucine, isoleucine and valine. The other amino acids are grouped in the fourth compartment. This part of the model assumes that each amino acid is present in the liquid phase and particulate phase *pro rata* of the protein fractions described above. Bearing in mind that the 4 amino-acid groups are treated in the same way, only the methionine compartment of the liquid and solid phases is described. The methionine compartment of the liquid phase (FMET) is supplied (RFMET, equation [10]) by the degradation of soluble proteins (equation [6]). This assumes that the level of methionine in the proteins from forage (IFMET) and concentrate (ICMET) are identical in the liquid and solid fractions:

$$RFMET = (ICMET \bullet C + IFMET \bullet F) K_{sn} \bullet SN \quad [10]$$

The flow into the small intestine from the FMET compartment occurs with the liquid phase. Methionine can be fermented by the microorganisms to give ammonia, volatile fatty acids (VFA) and adenosine triphosphate (ATP) (see later). The rate of fermentation, K_{metfe}, applied to FMET, results from a combination of publications (Chalupa, 1976; Rooke *et al,* 1984; Crooker *et al,* 1986; Varvikko, 1986; Susmel *et al,* 1989) in the form of a small data base. The rate corresponds to the rate collected in the publication with the closest diet and experimental design. The small number of results give a large range of variation (x 1 to x 3), which is apparently unexplainable. In addition, the part of the methionine that is 'free' or in the form of small peptides is used by the liquid-phase microorganisms (FMI) and incorporated directly into their own proteins. This flux of sampling is equal to the product of the growth of the free microorganisms (*d*FMI/*dt*), their protein concentration (MAA) and the level of methionine in these proteins (MMET). This quantity is also multiplied by a repartition factor, the variable *Cap*, which represents the proportions of methionine captured in relation to the microorganism requirement for methionine. Indeed the components of the microorganisms, in particular the proteins, are either captured directly and calculated as *Cap*•*d*FMI/*dt* or synthesised from carbon skeletons and ammonia and calculated as (1 – *Cap*)*d*FMI/*dt*. This principle of part synthesis/part capture applies to the microbial nitrogen as well as the carbohydrate constituents. *Cap* is an internal variable of the model which allows regulation of the entire system *via* microbial growth. It is therefore essential in the construction of the model. The amino acids captured are taken from the corresponding amino-acid compartment and do not supply ATP. The carbon skeletons and ammonia which serve in the *de novo* synthesis of microbial amino acids are taken from the VFA pool and the ammonia compartment. The reduction in VFA and ammonia used for the synthesis of amino acids is proportional to the number of moles of carbon present in each VFA compartment and to the number of moles of nitrogen necessary.

Modifications of the value of the coefficient *Cap* are associated with variations in the efficiency of microbial synthesis, $YATP_{max}$, since the cost of synthesis from captured nutrient precursors is not the same as that from newly synthesised precursors (see equation [29] later). As a consequence the rate of capture (*Cap*, equation [11]) is determined empirically in relation to the global efficiency of microbial proliferation, estimated by the relationship between microbial amino acids passing into the duodenum and carbohydrates degraded in the rumen EfAA (g microbial protein nitrogen/kg degraded carbohydrate) according to the work of Kristensen and Weisbjerg (1991). This relationship is directly inspired from the work of Leng and Nolan (1984), who proposed an equation for the efficiency of microbial growth (YATP: grams microbial dry matter/mole ATP) and the repartition of energy digested between the VFA, micro-

bial growth and the annex fluxes (methane and heat).

$$Cap = 0.16 + 0.0087\text{EfAA} \quad [11]$$

The evolution of the size of the compartments of 'free' methionine or small peptides is given by the equation [12]:

$$d\text{FMET}/dt = \text{RFMET} - K_l \bullet \text{FMET} - K_{metfe} \bullet \text{FMET} - Cap \bullet \text{MAA} \bullet \text{MMET} \bullet d\text{FMI}/dt \quad [12]$$

The 'free' methionine compartment of the solid phase (AMET) is supplied (RAMET, equation [13]) by the degradation of progressively degradable proteins:

$$\text{RAMET} = \text{ICMET} \bullet K_{cdn} \bullet \text{CDN} + \text{IFMET} \bullet K_{fdn} \bullet \text{FDN} \quad [13]$$

The passage into the small intestine occurs according to a rate that depends on the equilibrium between forage and concentrate in the diet. Because of the absence of specific results, the rate of fermentation used is the same as that used for liquid-phase methionine (K_{metfe}). Part of the methionine ($Cap \bullet \text{MAA} \bullet \text{MMET} \bullet d\text{AMI}/dt$, for $d\text{AMI}/dt$, see equation [30] later) is used directly by the attached microorganisms according to the global efficiency of microbial proliferation (equation [11]). Therefore the descriptive differential equation of the AMET compartment is:

$$d\text{AMET}/dt = \text{RAMET} - (K_{pc} \bullet \text{C} + K_{pf} \bullet \text{F})\text{AMET} - K_{metfe} \bullet \text{AMET} - Cap \bullet \text{MAA} \bullet \text{MMET} \bullet d\text{AMI}/dt \quad [14]$$

Carbohydrate fractions

The soluble carbohydrate contents of the rumen (SC, equation [15]) come from those found in the concentrate (ICSC) and forage (IFSC). They are degraded at a high rate by the free microorganisms in the rumen. The rate used (K_{sc}, l/h) is the same as that in the Cornell system (Sniffen *et al,* 1992). The soluble carbohydrates follow the liquid-phase flux into the small intestine.

$$d\text{SC}/dt = \text{DMI}(\text{IFSC} \bullet \text{F} + \text{ICSC} \bullet \text{C}) - K_{sc} \bullet \text{SC} - K_l \bullet \text{SC} \quad [15]$$

The insoluble starches are separated into 2 compartments FST and CST according to their origin: forage or concentrate. They are degraded by the attached microorganisms. The 2 compartments are constructed in the same way. For the forage, the starch that enters corresponds to the product of its proportion of starch (IFST) and its level of ingestion (DMI•F). The flow into the small intestine follows that of the forage particles at the rate of K_{pf}. The values used in the simulations for the rate of degradation, K_{fst} and K_{cst}, of the insoluble starch fractions correspond with the results grouped together by Sauvant *et al* (1994). The descriptive differential equations for these compartments are:

$$d\text{FST}/dt = \text{DMI} \bullet \text{IFST} \bullet \text{F} - K_{pf} \bullet \text{FST} - K_{fst} \bullet \text{FST} \quad [16a]$$

$$d\text{CST}/dt = \text{DMI} \bullet \text{ICST} \bullet \text{C} - K_{pc} \bullet \text{CST} - K_{cst} \bullet \text{CST} \quad [16b]$$

The potentially degradable cell-wall carbohydrates, based on *in sacco* measurements (Chapoutot *et al,* unpublished results), are separated into 2 compartments according to whether they come from the forage or concentrate. The structure of each compartment is identical. For the forage, the quantity of degradable cell-wall material entering is calculated from its level (IFCW) and the rate of passage into the small intestine is equal to that of the particles of forage (K_{pf}). The degradation of the cell-wall material is dependent on the activity of the microorganisms attached. This rate of degradation (K_{fcw} or K_{ccw}) is assumed to

be pH-dependent according to the Michaelis–Menten constant used by Sauvant *et al* (manuscript in preparation) from *in vitro* measurements. The pH is defined empirically from the ruminal concentration of VFA (equation [25] later).

$$K_{fcw}\ (\%/h) = K_{fcwmax} \bullet (pH - 5)/(0.5 + pH - 5) \quad [17a]$$

$$K_{ccw}\ (\%/h) = K_{ccwmax} \bullet (pH - 5)/(0.5 + pH - 5) \quad [17b]$$

In these equations, K_{fcwmax} and K_{ccwmax} represent the rates of potential degradation in optimal ruminal conditions. The values come from the literature obtained under these conditions. The differential equations describing the variations in the quantities of degradable cell-wall material are:

$$dFCW/dt = DMI \bullet F \bullet IFCW - K_{fcw} \bullet FCW - K_{pf} \bullet FCW \quad [18a]$$

$$dCCW/dt = DMI \bullet C \bullet ICCW - K_{ccw} \bullet CCW - K_{pc} \bullet CCW \quad [18b]$$

In the carbohydrate compartments, a fraction of the carbohydrate is fermented. However, part of this carbohydrate is captured and used directly in the synthesis of microbial biomass, without giving VFA (equation [27] later).

The undegradable cell-wall carbohydrates of the forage (IFUC) and concentrate (ICUC) transit without being transformed. The quantities entering and the rate of exit ($K_{pf} \bullet F + K_{pc} \bullet C$) depend on the composition and the level of ingestion of the diet. The evolution of the size of the undegradable cell-wall carbohydrate compartment (UC) is given by equation [19]:

$$dUC/dt = DMI(ICUC \bullet C + IFUC \bullet F) - (K_{pf} \bullet F + K_{pc} \bullet C)UC \quad [19]$$

Terminal fermentation products

The VFA come from the fermentation of carbohydrates and amino acids. Four categories of VFA are defined: acetate (AC), propionate (PR), butyrate (BU) and the branched-chain VFA (bVFA). As previously mentioned in recent models (Baldwin *et al*, 1987; Danfaer, 1990; Dijkstra, 1993) biases were introduced by the use of the stoichiometric relationships developed by Murphy *et al* (1982). A new method was therefore used to obtain more realistic values of the molar proportions of VFA. For this purpose a data base of literature results has been constructed which contains 130 results from 45 experiments in which the principle experimental parameter was the percentage of concentrate and where the molar proportions of VFA were measured (Lescoat and Sauvant, 1994; table I). The equations obtained are quadratic for acetate (equation [20]), propionate (equation [21]) and butyrate (equation [22]). The proportion of branched-chain VFA was calculated as the

Table I. Empirical regressions between ruminal VFA and the proportion of concentrate in the diet (Lescoat and Sauvant, 1994).

Y	*Model*	n	R^2	*RMSE*	*Equation*
Acetate	$66.97 - 21.1C^2$	129	0.79	3.95	20
Propionate	$15.83 + 20.6C^2$	129	0.8	4.17	21
Butyrate	$11.2 + 10.8C - 11C^2$	126	0.77	1.71	22

difference between 100% and the total of the 3 major VFA.

The mechanism of formation of VFA in the model involves a 2-step calculation. The fermented carbohydrates (RCHOVFA) and amino acids (RAAVFA) supply a flux of moles of 'glucose' with 6 carbon atoms which are partitioned between the different VFA. This partitioning is performed on the basis of the percentage of each VFA present in the rumen calculated from the proportion of concentrate (table I) weighted by the stoichiometric coefficient corresponding to each VFA obtained from a mole of 6 carbon atoms. More precisely, 1 mole of 6 carbon atoms can give, bearing in mind the production of CO_2, 2 moles of acetate, 2 moles of propionate, 1 mole of butyrate or 1 mole of branched-chain VFA. As a consequence, if table I gives the values of 65% acetate, 20% propionate, 12% butyrate and 3% branched-chain VFA in molar terms, the proportion of carbon chains of 6 carbon atoms which take the acetate fermentation pathway is (65/2)/((65/2) + (20/2) + 12 + 3). This allows the estimation of the quantity of ATP produced (see equation [26] later). For the amino acids, 1 mole fermented is assumed to give 1 mole of VFA, irrespective of the type.

The flux of VFA absorbed across the wall of the rumen ($RVFA_{ab}$, equation [23]) depends partly on the total quantity of VFA in the rumen and partly on the rate of absorption which is a Michaelis–Menten function of the ruminal VFA concentration ([VFA], mmol), and therefore of the pH.

$$RVFA_{ab} = VFA0.6/(1 + K_{vfa}/[VFA]) \quad [23]$$

with VFA = quantity of VFA in the rumen; K_{vfa} = 90 mmol, affinity constant.

The coefficients of this equation are taken from the model of Sauvant *et al* (manuscript in preparation). This approach is simpler than that of Dijkstra (1993). He took into account the influence of pH, as well as the level of VFA and each VFA was considered individually. Some of the VFA or intermediary carbon chains can be captured and contribute to the synthesis of microbial organic matter (Kristensen and Weisbjerg, 1991; Hvelplund, 1990) ($RVFA_{mi} = (1 - Cap)\bullet 0.9((dFMI/dt + dAMI/dt)/162)\bullet 6$ (in moles of carbon). Asumptions of 90% organic matter in the microorganisms and a microorganism 'molecular weight' of 162 g, equal to that of a mole of carbohydrate were made. For the bVFA, capture is supposed to be total since the microorganisms seem to be unable to synthesise the branched-chain carbon skeletons (Andries *et al*, 1987). The size of the VFA compartment is defined by equation [24]:

$$dVFA/dt(\text{mol of VFA}) = RCHOVFA + RAAVFA - RVFA_{ab} - RGVFA_{mi} \quad [24]$$

Ruminal pH is determined empirically from the concentration of VFA. The relationship used is calculated from the data base cited above according to an intra-experiment linear regression. The equation obtained is very close to that of Tamminga and van Vuuren (1988). The pH acts by negative feedback on the level of cell-wall degradation (equations [17a] and [17b]).

$$pH = 7.56 - 0.0131[VFA] \quad [25]$$
$$n = 57,\ R^2 = 0.80,\ RSD = 0.22$$

[VFA] = VFA ruminal concentration (mmol)

Intermediary molecules

ATP is the source of energy used for the maintenance and growth of microorganisms. The ATP produced is used if nitrogen is not limiting, which is the hypothesis of the model. The ATP that comes from the fermentation of soluble sugars or free amino acids is used for the maintenance and the growth of the microorganisms in the liquid phase. The degradation of the particulate carbohydrates and proteins forms ATP,

which is used by the microorganisms attached. The stoichiometric coefficients of the formation of ATP from different metabolic pathways are those proposed by Russell and Wallace (1988). Equation [26] calculates the quantities of ATP produced from the daily flux of VFA production in moles.

$$\text{ATP (mol/d)} = 2.5\text{AC} + 1.75\text{PR} + 3.5\text{BU} \quad [26]$$

Some of the carbohydrates degraded do not give ATP because their intermediary products are directly captured by the microorganisms for biomass synthesis. This flux is substracted from the flux available for the production of VFA and ATP. Thus for the microorganisms attached the fraction RGL_{ami} (equation [27]), expressed in moles of carbohydrate, depends on the *Cap* (equation [11]) and on the quantity of microorganisms formed *d*AMI/*dt*.

$$RGL_{ami}(\text{mol carbohydrate}) = Cap(d\text{AMI}/dt)/162 \quad [27]$$

Ammonia (NH_3) is a terminal product of the fermentation of amino acids (RAA) and urea nitrogen in the ration. It is absorbed across the rumen wall according to the law of mass action, the level of absorption being K_{NH_3ab} (Sauvant, 1992b). K_{ab} is adapted from the model of Sauvant (1992b) and has a value of 0.01/h/%CP. The ammonia can also be used as a nitrogen source for the growth of the microorganisms and its incorporation (R_{NH_3mi}) is dependent on the rate of capture (*Cap*, equation [11]).

$$dNH_3/dt = \text{RAA} - K_{NH_3ab} \bullet NH_3 - R_{NH_3mi} \quad [28a]$$

$$R_{NH_3mi}(\text{mol}) = (1 - Cap)\text{MAA}(d\text{FMI}/dt + d\text{AMI}/dt)/115 \quad [28b]$$

$$K_{NH_3ab}\ (/\text{h}) = K_{ab} \bullet \text{CP} \quad [28c]$$

CP: total crude protein in the diet (%)

The molecular weight of amino acids is assumed to be 115 g.

The microorganisms

Two microorganism compartments are defined: those fixed to the solid particles (attached microorganisms (AMI)) and those which are in suspension in the liquid phase of the rumen (free microorganisms (FMI)). Their dynamic passage into the intestine is linked to the phase in which they are present. The compartment size is expressed in terms of dry matter and their energy source comes from the breakdown of the degradable fractions of their associated constituents and the flux of resulting ATP. The 2 microbial compartments are constructed in a similar fashion, and as a consequence only the attached microorganism (AMI) compartment is described.

The average composition of the microorganisms (table II) is taken into account, and in particular their amino-acid profile (table III; Hvelplund and Madsen, 1989; Le Hénaff, 1991). These microorganisms pass into the small intestine with the particles of the diet. The microorganisms have an energy requirement for maintenance proportional to the size of their compartment and equal to K_{mia} = 0.0016 mol ATP/g AMI/h (Isaacson *et al*, 1975; Demeyer and van Nevel, 1986). The costs of microbial dry matter synthesis (Church, 1988, table IV) depend on both its composition (each constituent has a different cost of synthesis (Belaich, 1986) and

Table II. Microbe composition (% of dry matter).

Component	*% dry matter*
Amino acid (AA)	MAA
Mineral matter (MM)	10
Non-amino-acid N (NPN)	6.25N – MAA
Lipids and carbohydrates (LC)	100 – 10 – 6.25N

Table III. Microbe amino-acid profile (% AA) (Le Hénaff, 1991).

Amino acid	*%*
Leucine	7.7
Isoleucine	5.9
Valine	6.8
Lysine	8
Methionine	2.5

its rate of capture *(Cap)*, which determines the quantities synthesised (high cost) or captured (low cost) (equations [11] and [29]). The efficiency of microbial use of ATP for growth ($YATP_{max}$) is calculated from these costs (table IV) and from the composition of the microorganisms (table II). $YATP_{max}$ is calculated by multiplying the cost of synthesis (SMC, table IV) of each molecule by the quantity of each molecule synthesised by the microorganisms and by adding the cost, which is lower, of polymerisation of the molecules that corresponds with the fraction captured (*Cap*, equation [11]). The quantity of microorganisms formed per mole of ATP is obtained from this calculation. The abbreviations used are given in table II, for the composition, and table IV, for the costs. If X is a constituent of the dry matter, XC is its cost of synthesis.

Table IV. Synthesis cost of microbe components (adapted from Church, 1988).

Component	*Cost (mmol ATP/g)*
Small molecule (SMC)	10
Lipid (LC)	25
Carbohydrate (CC)	25
Non-amino-acid N (NAANC)	20
Mineral matter (MMC)	20
Amino acid (AAC)	50

$$YATP_{max}(gAMI/molATP) = 1\,000/(Cap(LC + NAAN + MM + MAA)\,SMC + (1 - Cap)(LC(LC + CC)/2 + NAAN \bullet NAANC + MM \bullet MMC + MAA \bullet AAC)) \quad [29]$$

The differential equation [30], which gives the evolution of the size of the attached microorganism compartment, gives the balance between net growth and the exit flux to the small intestine.

$$dAMI/dt = YATP_{max}(RAT_{ami} - K_{mia} \bullet AMI) - (K_{pf} \bullet F + K_{pc} \bullet C)AMI \quad [30]$$

Exit parameters of the model

Simulations allow values at dynamic equilibrium to be obtained for all the parameters of the model. This information is accessible throughout the calculation and can be summarised for part of the time of simulation.

The coefficients of ruminal feed digestibility represent simple criteria indicating the result of the activity of the rumen. They allow the verification of the functional coherence of the model with values given in the literature. The duodenal flux of carbohydrates leaving the rumen is the sum of the exits from the soluble carbohydrate, starch, degradable carbohydrate and undegradable carbohydrate compartments. The distinction between these different types of carbohydrates allows the validation of different simulated post-ruminal digestibilities according to the carbohydrate fraction. The efficiency of microbe growth (E_{fmi}) corresponds in general in the literature to the ratio of microbial nitrogen passing into the duodenum (Nm) over the quantity of organic matter truely degraded in the rumen (tOMD) (g Nm/kg tOMD).

The daily quantities of total VFA absorbed allows the calculation of available

energy for the intermediary metabolism of the animal.

The flux of dietary proteins that transits to the duodenum corresponds with the sum of the exits from the protein and amino-acid compartments of the rumen. The distinction can be made between soluble, degradable and undegradable proteins and different amino acids in the duodenum. The proteins and dietary amino acids really absorbed (PDIA) are calculated using coefficients of true digestibility from the PDI system (INRA, 1988). The fluxes of absorbed proteins in the intestine of microbial origin (PDIM) are calculated from the duodenal fluxes of microbial protein multiplied by the true digestibility coefficient of the PDI system, 0.8. The values for digestible methionine and lysine (MetDi, LysDi) in the diets are obtained by calculating the ratio between methionine or lysine absorbed in the small intestine and the proteins absorbed in the small intestine limited by energy, PDIE.

Certain parameters are necessary if the model is to function correctly and these are indicated in table V. The model can be represented in the form of a diagram containing 22 compartments (fig 1).

RESULTS

Results of internal validation. Analysis of the sensitivity to initial conditions and parameters

The dietary regimen used in these studies of sensitivity corresponded to a simple complete diet which included, on a dry matter basis, maize silage (70%) and soya bean meal 48 (30%). The animal used was a cow producing 35 l of milk and which ate 20.45 kg dry matter per day. The rates of degradation of the different dietary fractions came from INRA results (1987) and Chapoutot *et al* (unpublished results). The composition of the microorganisms came from Hvelplund and Madsen (1989) and Le Hénaff (1991). Feedstuff compositions were derived from the data bank of animal feeding of the AFZ (AFZ, 1993).

Five groups of parameters were chosen for this study to cover the large range in variations of published values (basal values in brackets): the rate of exit from the rumen (RO, K_l, 0.086/h; K_{pf}, 0.05/h; K_{pc}, 0.065/h), the rate of absorption of VFA (VFAA, 0.6/h), the efficiency of ATP production from fermentation (ATPP), the level of microbial amino acids (MAA (32% of the dry matter)) and the maintenance energy requirement of the microorganisms (K_{mi}, 0.0016 mol ATP/g microorganisms). The consequences of these variations were considered on 4 parameters: real rumen digestibility of organic matter (tOMD, 57.75%), growth efficiency of rumen microorganisms (ME, 31.92 g mN/kg OMD), PDIE value of the diet (2 546 g/d) and ruminal pH (6.08).

The principle of sensitivity exploration was used to test the influence of variations in the basal value for each parameter, ± 25%. The study was conducted according to a complete factorial experiment 2^5. The use of such a factorial design allows the study not only of principle effects but also of interactions between factors. This is not possible if each factor is treated independently (Kobilinsky and Monod, 1991). The method is useful because it shows when effects are more than proportional between the factors studied. The results were analysed by multiple regression models containing significant effects. The calculations were performed using GLM of SAS (SAS, 1988). Only the responses of the principle effects were considered as well as first-order interactions with a sufficiently high Fisher test value, bearing in mind that the effects were all significant at the 1% level. The results indicated (table VI) correspond to a variation of 25% above the basal value includ-

Table V. Definition of input parameters.

Parameter [a]	*Description (origin)* [b]	*Compartment* [c]
Animal description		
LW	Live weight (kg) (P)	All
MP	Milk production (kg/d) (P)	All
Diet composition		
F	Proportion of forage (%) (P)	All
IJSN	Proportion of soluble N (P or AFZ, 1993)	SN
IJDN	Proportion of degradable N (P or AFZ, 1993)	JDN
IJUN	Proportion of undegradable N (P or AFZ, 1993)	UN
IJAAi	Proportion of amino acid (P or AFZ, 1993)	FAAi; AAAi
IJSC	Proportion of soluble carbohydrate (P or AFZ, 1993)	SC
IJST	Proportion of starch (P or AFZ, 1993)	JST
IJCW	Proportion of degradable cell wall (P or AFZ, 1993)	JCW
IJUC	Proportion of undegradable carbohydrate (P or AFZ, 1993)	UC
Degradation and fermentation rates		
K_{sn}	Soluble N degradation rate (P, INRA, 1988; Sniffen *et al,* 1992)	SN; FAAi
K_{jdn}	Degradable N degradation rate (P, INRA, 1988; Sniffen *et al,* 1992)	JDN; AAAi
K_{aaife}	Amino acid fermentation rate (review)	FAAi; AAAi; VFA
K_{sc}	Soluble carbohydrate degradation rate (P, INRA, 1988, Sniffen *et al,* 1992)	SC; VFA
K_{jst}	Starch degradation rate (P, INRA, 1988; Sniffen et al, 1992)	JST; VFA
JV_{max}	Maximal rate of cell-wall degradation (P, INRA, 1988; Sniffen *et al,* 1992)	JCW; VFA
Microbes		
M_{aa}	Microbe amino-acid content (P, Lescoat *et al,* 1994)	FMI; AMI
K_{mi}	Microbe maintenance cost (mmol ATP/g/h) (Isaacson *et al,* 1975)	FMI; AMI

[a] J = C (concentrate) or F (forage); [b] P: the information is provided by the publication; [c] compartment influenced by the parameter.

ing the interactions. The sensitivity of the statistic models used means that only the magnitude of the results is interpretable.

The sensitivity analysis shows some strong parameter influences on the output of the model. The ruminal digestibility of organic matter is above all affected by the rate at which digesta leaves the rumen. The growth efficiency of the microorganisms also increases strongly under the influence of transit time. It also increases with the efficiency of ATP formation and with the level of microbial nitrogen, however, the efficiency is reduced by the maintenance requirement. The total flux of PDIE increases greatly with transit rate, the production of ATP, the level of microbial nitrogen and the absorption of VFA. The flux is reduced by the maintenance requirement of the microorganisms. Ruminal pH is only slightly influenced by the above factors except the rate of absorption of VFA.

In the factorial 3^2 plan, the influence of the lysine and methionine profile of the microorganisms on the PDI profile of the same amino acid was studied. The 3 levels studied were the basal level and +25% and −25% of the basal level. The results

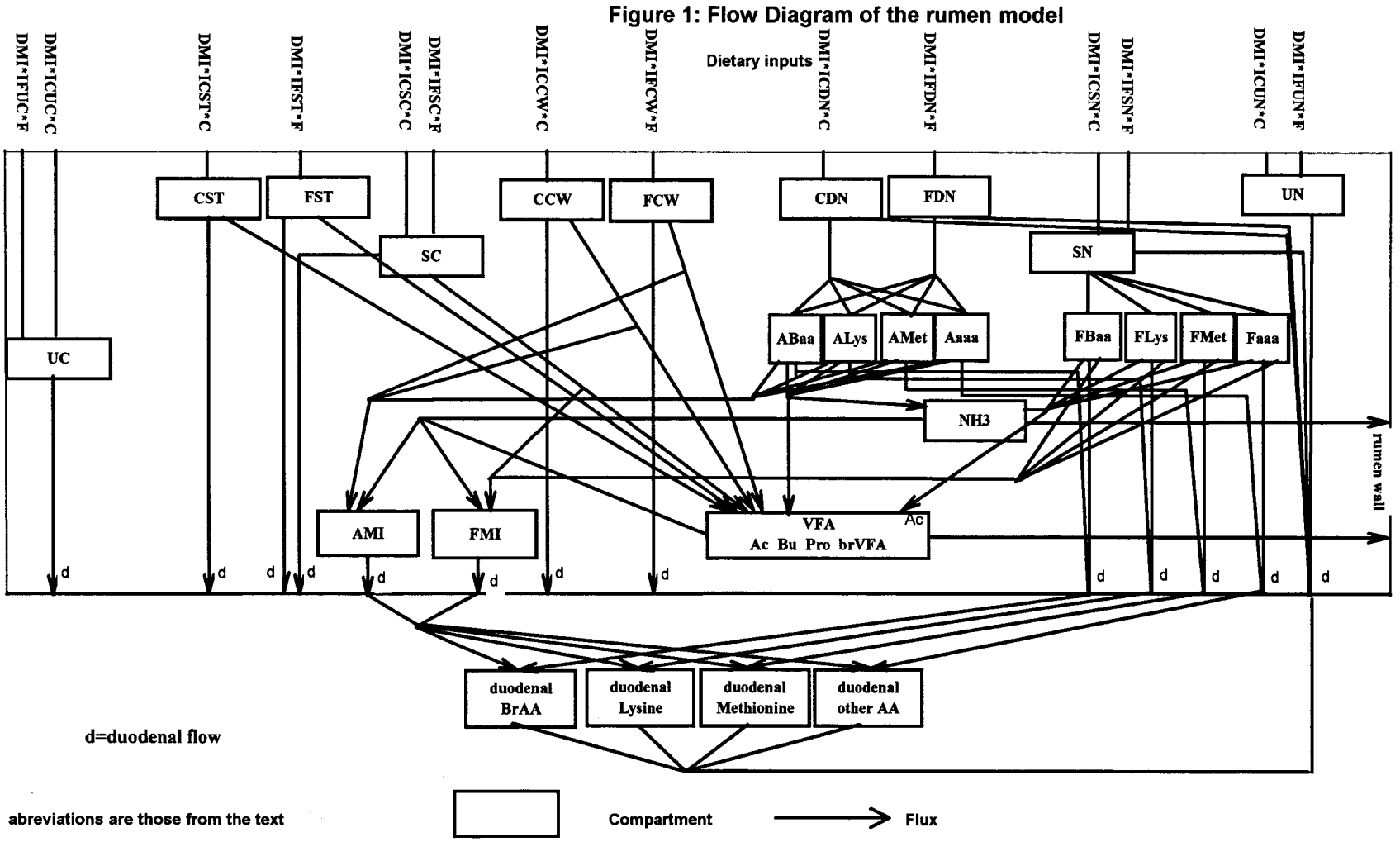

Fig 1. Flow diagram of the rumen model (see abreviations in the text).

Table VI. Sensitive analysis [a] model response to a 25% increase in main effect and first-order interaction.

Unit	*tOMD (%)*	*ME (gmN/kg OMD)*	*PDIE (g/d)*	*pH*
Initial value	57.75	31.92	2 546	6.08
RO	–4.35	4.22	173	0.15
ATPP	1.06	7.41	235	0.06
K_{mi}	–0.52	–3.46	–112	–0.04
MAA	0.64	7.00	215	–0.03
VFAA	0.98	NS	17	0.26
RO.ATPP	NS	0.96	NS	NS
RO.K_{mi}	NS	NS	21	NS
RO.MAA	0.19	0.88	NS	NS
RO.VFAA	–0.32	NS	NS	NS
ATPP.K_{mi}	NS	–0.77	–23	NS
ATPP.MAA	0.27	1.56	44	NS
ATPP.VFAA	–0.16	NS	NS	NS
K_{mi}.MAA	NS	–0.73	–20	NS

[a] RO: outflow rate from the rumen; ATPP: ATP production; K_{mi}: microbe maintenance cost; MAA: microbe amino-acid content; VFAA: VFA absorption rate through the rumen wall. NS: no significant difference compared to a relative Fisher test.

obtained show that the levels of microbial methionine and lysine influence strongly and linearly the proportions of digestible lysine (LysDi, %PDIE) and methionine (MetDi, %PDIE) in the diet. The variations are explained by a scale effect: if the level of microbial methionine is increased by 25%, this represents about 70% of the methionine measured in the duodenum and corresponds to a 70%•0.25 increase in MetDi. There is no apparent interaction between the level of lysine and that of methionine.

Results of external validation

Presentation of the results

Validation of general criteria such as ruminal digestibility and microbial efficiency have been performed on experimental results (from the PhD of Archimède, 1992) obtained in goats at maintenance. These results (table VII) were used because of the diversity of the 14 diets tested and the large number of measures made. In addition the results were not used to establish the model. The quantities of dry matter ingested, the chemical compositions of the diets, and the degradation parameters measured *in sacco* of the different fractions of the diets were all used for the simulations. The part of the model which concerns the amino acids was removed so that only digestive parameters were studied. The model used for these validations has the same structure as the general model except that the amino-acid compartments were removed.

The ability of model prediction is estimated using the same procedure as Dijkstra (1993). The error percentage is defined by the ratio between the root mean square of the prediction error (MSPE) over the average of the results observed. The MSPE is separated into 3 fractions which represent the overall bias of prediction (OBP), the devi-

Table VII. Comparison of observed and simulated results for 14 diets (Archimède, 1992).

Variable	*tOMD (%)*		*pH*		*[VFA] (nmol)*		*%C2*		*Microbial efficiency (g mN/kgOM)*		*[NH_3] (mg)*	
	obs	*simu*	*obs*	*simu*	*obs*	*simu*	*obs*	*simu*	*obs*	*simu*	*obs*	*simu*
MS0 [a]	34.4	64.1	6.86	6.91	67.7	48.4	74.7	69.9	15.3	19.1	61.5	–35
MS/FS30 [a]	54.6	57.9	6.61	6.69	81.9	65.5	69	67.4	10.8	26.4	156	50.2
MS/FS60 [a]	53.1	53.4	6.53	6.56	72	75.3	64.7	61.3	12.5	29.4	189	153.6
MS/SS30 [a]	42.7	47.7	6.52	6.91	75.1	48.9	71.8	67.4	14.8	28.3	130	9
MS/SS60 [a]	39.1	48.1	6.51	6.71	71.8	64.2	67.7	61.3	14.1	27.9	169.6	67
MS/CW30 [a]	50.2	59.3	6.52	6.85	88.7	53.4	72.1	67.4	13.7	23.1	142.7	61.4
MS/CW60 [a]	55.3	44.6	6.72	6.8	80.1	57.5	65.4	61.3	12.9	27.18	218.1	146
AH0 [a]	45.4	70.3	7.01	6.67	105.1	67	72.5	69.9	13.4	24.8	300	254
AH/FS30 [a]	48.3	63.4	6.93	6.58	96.1	73.2	69.1	67.4	14.1	28.06	287.6	264
AH/FS60 [a]	59.4	57.8	6.63	6.41	105.7	87	65.4	61.3	10.5	28.5	346.8	315
AH/SS30 [a]	45.1	58.5	6.81	6.69	101.2	65.5	69.7	67.4	14.1	27.5	294	220
AH/SS60 [a]	49.6	47.9	6.66	6.52	91.1	78.2	65.6	61.3	14.3	32	236.7	235
AH/CW30 [a]	49.1	62.6	6.92	6.83	97.4	55	72.3	67.4	12.5	26.8	273.1	186
AH/CW60 [a]	46.7	50.3	6.59	6.58	103.7	74.1	66	61.3	17.1	31.6	302.4	299
MSPE (%) [b]		27.4		3.2		29.6		5.91		103		33
OBP (%) [b]		37		0		78		89.5		93		73
DRS (%) [b]		40		40		3		1.5		5		20
DP (%) [b]		23		60		19		9		2		7

[a] MS: maize stover; AH: alfalfa hay; FS: concentrate with fast starch; SS: concentrate with slow starch; CW: concentrate with cell-wall material; number: concentrate percentage in the diet. [b] MSPE: mean square predicted error; OBP: overall bias of prediction; DRS: deviation of the regression slope from one; DP: disturbance proportion.

ation of the regression slope (DRS) in relation to 1 and the disturbance proportion (DP) (Bibby and Toutenburg, 1977). Breakdown of the MSPE allows the study of the behaviour of the model and the types of biases obtained. These results allow modification of the model by showing phenomena not previously taken into account.

The simulated values of ruminal digestibility of organic matter were different but they were of the same order of magnitude. However, the apparent ruminal digestibility of organic matter was not accurately simulated. The MSPE was 27.4%, and can be separated into OBP 37%, DRS 40% and DP 23%.

Simulated ruminal pH followed the same variation as the measured pH for the lucerne–hay-based-diet. The same result is observed for diets based on maize stems. However, maize–stem-based diets which include slowly digested starch and fibre concentrates did not follow the same variations, since the simulated pH was higher. For this latter case, the ranges of pH did not have a strong influence on microbial activity and the simulation can therefore be considered correct. The MSPE was 3.2%. The ruminal concentration of VFA was underestimated by the model, however, the simulated values are of the same order of magnitude. The MSPR was 29.6% but it was comprised of essentially OBP (78%), which is probably linked to a poor estimation of absorption. On the other hand, the hierarchy for the VFA concentrations was not the same. The molar proportions of acetate were systematically underestimated (3%) by the model but the relationship between estimated and measured values was MSPE 5.9% and OBP 89.5%.

The simulated microbial efficiency was overestimated by 100% of the measured values, with consequently an MSPE of 103%. This overestimation was also found in the decomposition of the error, with an OBP of 93%. However, given the difficulties involved in measuring microbial efficiency *in vivo*, such a difference is probably acceptable. In addition, the rumen microorganisms of these goats were low in nitrogen (about 5.5% N, Archimède, personal communication) and the level used in the model is 7.1%. The ruminal concentration of ammonia was systematically underestimated by the model with an RMSPE of 33%, but the hierarchy of the diets was maintained. This underestimation gave an OBP of 73%. The difference could be due to an incorrect estimation of ruminal absorption of ammonia or an overestimation of ammonia nitrogen fixation by the microorganisms.

The model has been tested for its capacity to take into account the influence of the level of ingestion and the quantity of concentrate ingested on microbial efficiency, on the digestibility of organic matter and on the pH in order to compare qualitatively these values with published results. The simulated diets had the same composition as that used for the internal validation. For a level of dry-matter ingestion which increased from 1 to 15 kg, organic matter digestibility was reduced by about 10% and pH by 1%. This result is similar to that obtained by Robinson *et al* (1986). In addition, microbial synthesis efficiency increased by about 8%, which is in general agreement with the empirical regression calculated by Ramangasoavina and Sauvant (1993). The responses to the proportions of concentrate ingested give results quite similar to observations in the literature. However, it appears that there is an overestimation of microbial synthesis efficiency when compared with the result from the regression of Ramangasoavina and Sauvant (1993) for extreme diets containing little or a lot of concentrate.

Presentation of the results on amino-acid flux

The results from various publications (Merchen and Satter, 1983; Rooke *et al,*

1983; Stern *et al,* 1983, 1985; Prange *et al,* 1984; Chamberlain *et al,* 1986; Titgemeyer *et al,* 1988; Windschitl and Stern, 1988; Zerbini *et al,* 1988; MacCarthy *et al,* 1989; Waltz *et al,* 1989; Klusmeyer *et al,* 1990; Aldrich *et al,* 1993) and from unpublished experiments (Schwab *et al*) were used to test the validity of the model to predict the flux of lysine and methionine passing into the duodenum in cattle. The publications were used if they contained enough parameters to be adapted to the model. They cover a large range of diets and types of bovine. The number of duodenal fluxes used and simulations was 49.

The duodenal flux of methionine (fig 2) and lysine (fig 3) were on average correctly estimated by the model. Linear regression of the measured and predicted fluxes gives an ordinate which is not different from zero ($P > 0.05$). For the 49 fluxes, the predicted lysine flux was underestimated by 1.3% ($R^2 = 0.98$) compared with the measured flux and the estimated methionine flux was

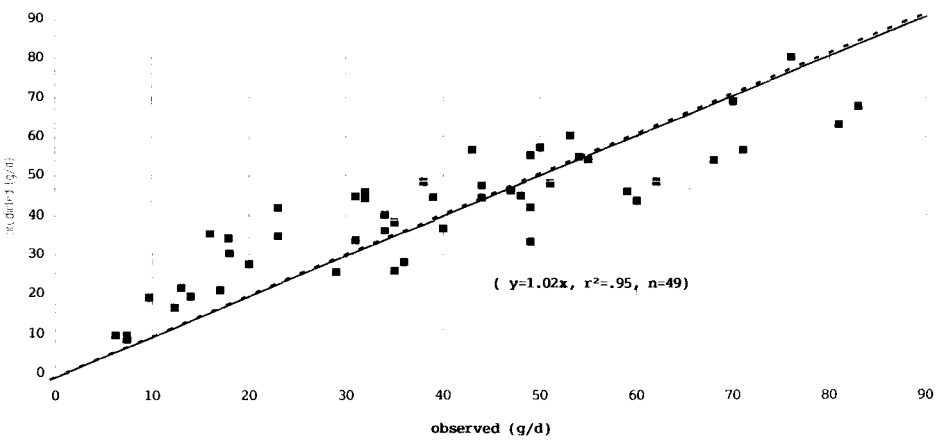

Fig 2. Duodenal flow of methionine.

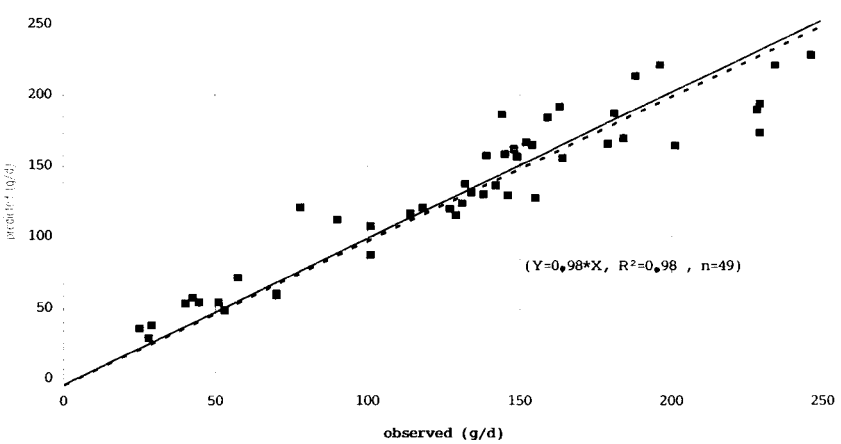

Fig 3. Duodenal flow of lysine.

over-estimated by 1.2% (R^2 = 0.947) compared with the measured flux. The residual standard deviation was 10.2 g for the methionine and 19.8 g for the lysine. MSPE was 28.8% for methionine and 21.8% for lysine. Breakdown of the errors gave a DP of more than 95%.

Levels of digestible lysine (LysDi, %PDIE) and methionine (MetDi, %PDIE) in the PDIE have been published by Rulquin *et al* (1993) for the raw materials given in the INRA tables (INRA, 1988). The model has been adapted to enable the evaluation of LysDi and MetDi of 6 raw materials, simulating diets composed of a single raw material. The results obtained were satisfactory. The model discriminates between the 6 diets and gives a range of LysDi and MetDi values which are of the same magnitude as those proposed by Rulquin *et al* (1993, table VIII).

DISCUSSION

The mechanistic models published in the last 10 years concerning rumen digestion (France *et al,* 1982; Baldwin *et al,* 1987; Danfaer, 1990; Russell *et al,* 1992; Dijkstra, 1993) are similar in the sense that they all integrate substrate, microorganism and terminal product compartments. The principle differences between these models concern the degree of accumulation of knowledge applied to each of the 3 compartments. The present model has been constructed, as have earlier models, to give values for the average dynamic equilibrated state during the nycterohemeral period. It has not been tested in unequilibrated conditions, which would require the integration of certain structures having fundamental dynamic actions (temporary storage of polysaccharides, compartmentalisation of protozoa, *etc*) and would also require the integration of possible short-term variations in some fluxes (variations in transit, absorption and recycling during the post prandial phase, delay phenomena, *etc*). This approach, apart from equilibrated situations, has undergone preliminary investigation (Dijkstra, 1993) but published validations only concern values at equilibrium.

The definition of the dietary fractions taken into account in the mechanistic model of the rumen is the result of a compromise between the wish to have criteria allowing a full explanation of the quantitative variations in digestive processes and the necessity to use simple and inexpensive analyses. The proposed model for these characteristics is simpler than that of Baldwin *et al* (1987) and Danfaer (1990); but the level of definition is comparable to that of Dijkstra (1993). The classic chemical analyses and the measurements of cell-wall material of van Soest,

Table VIII. Comparison between simulated and INRA values of MetDi and LysDi (%PDIE).

Feedstuff	*INRA number* [a]	*MetDi model*	*MetDi INRA*	*LysDi model*	*LysDi INRA*
Maize silage	428	2.21	1.98	6.38	6.92
Barley	616	2.35	1.88	7.02	6.83
Soyabean meal	676	1.66	1.52	6.83	7.01
Fish meal	702	2.59	2.76	7.21	7.82
Gluten meal	634	2.41	2.48	1.94	3.71
Grass	140	2.32	1.93	8.13	7.16

[a] INRA (1988).

and of starch and soluble sugars are used in all laboratories of feedstuff analysis. The amino-acid profile analyses are also performed in series by some laboratories, the results being more variable and obtained at a higher cost than the previously mentioned analyses (Mossé, 1990). However, methods that use nylon bag measurements necessitate methodology which is much more difficult and are therefore used mainly in research. In addition, even though there have been many publications in recent years on the profile of the degradation of nitrogen, there is much less information available on the evolution of the amino-acid composition of the non-degraded fraction. The situation is similar for the profiles of degradation of starch carbohydrates and plant cell-wall material. Finally, the coherence between the criteria of degradation *in sacco* and ruminal digestibility has been questioned. Indeed it seems that the nylon bag method can greatly underestimate the levels of degradation of carbohydrate fractions which are present in lower quantities (Archimède, 1992). Therefore the accuracy of the results provided by the model depends on the nature of the initial data used.

The variations in particle and liquid-phase transit have been largely ignored by published complete models (Black *et al,* 1980; Baldwin *et al,* 1987; Danfaer, 1990). These aspects have been considered in a partial ruminal model (Mertens and Ely, 1979) but the influence of factors of variation such as feeding and concentrate level were not integrated. The model proposed by Dijkstra (1993) used multiple regressions from the suggestions of Owens and Goetsch (1986) and has been partly validated by the results of Robinson *et al* (1986). The empirical relationships that are used in the present model have been established (Sauvant and Archimède, 1989) on a larger number of results than Owens and Goetsch (1986). Even though the amplitude of the residual variations of these equations are large, the results from the simple validation that we have performed to study the influence of different combinations of dry matter intake and proportions of concentrate on ruminal digestion and microbial growth efficiency are for the most part satisfactory. These equations have been adapted and used in the Cornell system (Chalupa *et al,* 1991). In addition, the results of transit flux appear to correspond with the equation for the prediction of ruminal volume proposed by Rémond, 1988.

The microorganisms include many subpopulations which mutually interact (Fonty, 1990; Prins, 1990; Stewart, 1990). The model proposed groups together present knowledge by only considering fixed and free microorganisms using a distinction frequently used during recent years (Merry and MacAllan, 1983). The attached microorganisms degrade indiscriminately insoluble, starch and cell-wall carbohydrates. Other rumen models use many sub-groups of microbes. Indeed Baldwin *et al* (1987) and Dijkstra (1993) separated cellulolytic and amylolytic microorganisms. Dijkstra also took into account a protozoa compartment which interacted with the amylolytic bacteria, while Danfaer (1990) only took into account 1 microbial compartment even though his model was very detailed for most ruminal digestive mechanisms. In the model presented the separation between the 2 microbial compartments assumes that there is no possibility of exchange or interaction even if experimental observations suggest that there are exchanges and that there is a close relationship between microorganisms, from strongly attached to totally free particles (Yang, 1991). In addition the chemical and analytical composition of the microorganisms is assumed to be identical in the 2 populations. This is however not true and many studies have shown that there are differences in composition between the 2 groups. The level of nitrogen used in the model for the microbes is an average taken from the literature (Lescoat *et al,* 1994) but it varies according to the phase studied

(Merry and MacAllan, 1983), the method of isolation (Storm and Orskov, 1983) and time of sampling in relation to the meal (Jouany and Thivend, 1972; Czerkawski, 1976; Yang, 1991). The amino-acid profile of the microorganisms used is equally open to discussion because of the poor repeatability of the analyses and the lack of a reliable microbe isolation technique (Poncet, personal communication). As a result the coefficient of variation of the values measured for methionine in microbial protein is 25% and for lysine 12% (Le Hénaff, 1991; Clark *et al,* 1992). These phenomena reduce the precision of predicted amino-acid flux into the duodenum.

The microbe growth, permitted by the ATP available above maintenance requirements, is highly dependent on the efficiency ($YATP_{max}$) which varies according to the parameter *Cap* (equation [11]). The model assumes that the cost of polymerisation is low (CPM, table IV) and that the cost of synthesis of microorganism monomers is high (table IV). However, Belaich (1986) states that the cost of monomer synthesis is much lower than that for polymerisation. The evaluation of the efficiency $YATP_{max}$ of the model can therefore be criticised even if the value obtained is within the range of values proposed in the literature. In order to study the importance of this factor, a sensitivity analysis was performed. The results show that better knowledge of this factor is needed, both for its principle effect and its interactions. A more mechanistic approach for microorganism growth is necessary namely to take into account the variations in microorganism composition and the existence of microbe populations with different behaviours in the presence of the same substrate. In the model proposed by Black *et al* (1980), they attempted to take into account the differential efficiencies of the microbial constituents on the basis of biochemical equations proposed by Reichl and Baldwin (1975). These equations should be recalculated using more recent data. In addition, the efficiencies obtained can depend greatly on the precursor molecule. The values proposed by Church (1988) and reused in the present work do not assume the same efficiencies as Black *et al* (1980) for fat and mineral fractions. More recently, Dijkstra (1993) has similarly integrated various costs of synthesis for the microbial components. He has also taken into account the variation in the level of microbial carbohydrate reserves. The variations in the profile of nitrogen-containing nutrients used by rumen microorganisms are in the present case linked mainly to their growth efficiency (or coefficient *Cap*). Baldwin *et al* (1987) and the Cornell model (Russel *et al,* 1992) have taken into account the existence of a minimum amount of nitrogen-containing nutrients in the form of amino acids. This principle was not used in the present model due to the small number of experimental results available to justify the quantitative adoption of this hypothesis.

The values used for the maintenance energy requirement of the microorganisms are similar to those of previous models (Baldwin *et al,* 1987; Danfaer, 1990; Dijkstra, 1993). In fact the estimations of this requirement varies within a large range of values (Belaich, 1986; Demeyer and van Nevel, 1986). However, it should be borne in mind that most of these available data result from *in vitro* studies and that their application for *in vivo* use is problematic. In this context it is justifiable to use the same value of maintenance for attached and free microbes. Nevertheless, Russell *et al* (1992) have defined different requirements for maintenance according to the source of energy. It is possible that there is a higher maintenance requirement for free microbes because of a high level of capture of bacteria by protozoa which probably increases the energy need (Ushida *et al,* 1991). The incertitude concerning these values encouraged us to study the specific sensitivity of these parameters. As for the efficiency

$YATP_{max}$, the importance of maintenance needs in the determination of duodenal flux is emphasized and an approach more closely focused on the breakdown of the microbial compartments is an interesting idea to use to explain part of the variability observed for the maintenance requirements and in consequence the fluxes.

Published rumen models have not tried to study digestive interactions. The model proposed takes into account the possibility of a negative feedback effect of pH on cellulolysis. However, the validations performed to date have not focused on this aspect. Moreover, the model does not integrate the influence of microbial compartment size on the flux of degraded substrates unlike the models of Baldwin *et al* (1987) and Dijkstra (1993). In order to take into account the digestive interaction phenomena that does not concern only the cell-wall material (Archimède, 1992), it is necessary to first integrate this increase in microbe populations. In addition, the phenomena of competition between the different species should be taken into account by limiting ruminal microorganism colonisation to a maximum which corresponds to a limitation in the size of the possible contact surface between substrate and microbes (Yang, 1991). Finally, it would be probably necessary to add other physicochemical parameters of rumen ecology (osmotic pressure, ratio $NADH_2$/NAD *etc*).

The models published up to now have used the stoichiometric equations of Murphy *et al* (1982) in order to predict the different quantities of VFA produced. Many authors, in particular Baldwin *et al* (1987) and Dijkstra (1993) have underlined that the use of these equations was probably at the origin of faults in the validation of their models. For this reason we have tried to use a method allowing direct integration of the known influence of the proportion of concentrate in the diet on the stoichiometry of fermentation. As a consequence, external validation was performed on the production of VFA using the data from the literature which had not been previously used. The relationships that we have developed represent in fact an important simplification in relation to the diversity of responses observed in the literature, particularly for diets containing more than 50% concentrate. Better understanding of this diversity is needed in order to integrate it into the model, if possible in a mechanistic way. Moreover, these relationships assume, as did Murphy *et al* (1982), that there is a direct relationship between the production and the concentration of VFA in the rumen. This type of relationship has been rejected by Dijkstra (1993).

The model of Dijkstra (1993) is without doubt the most complete and precise in relation to the influence of different factors on the absorptive flux of the VFA. Our proposal is simpler and makes use of that of Sauvant *et al* (manuscript in preparation) and gives very similar results for the total flux of VFA absorbed. However, the equations of Dijkstra (1993) assume lower absorptive flux for acetate but higher flux for propionate and butyrate.

The model contains many parameters determined in an uncertain way. Internal validations show that these poorly understood parameters play an important role in ruminal digestion. Indeed variations of ±25% in the rate of flow have an important influence on rumen digestibility of organic matter and the efficiency of microbial growth. It is important to remember that the adjustment of literature values obtained from looking at the influence of ingestion level and proportion of forage on transit time give coefficients of the residual variation of 18.4, 19 and 24.2% for the flows K_{pf}, K_{pc} and K_l, respectively (Sauvant and Archimède, 1989). Similar comments can be made for the formation of ATP per mole of substrate fermented which varies within the range 3 to 6 according to the authors. Also mainte-

nance requirements can vary between 0.00021 to 0.0189 mole ATP per gram of microorganism per hour (review Belaich, 1986). The results of these sensitivity analyses and the large ranges of variation emphasize the necessity to improve the precision of our understanding of the relationships which associate the level of ingestion, transit, ruminal digestibility and microorganism growth, without forgetting the composition of the biomass and its maintenance needs. Another advantage of internal validation has been to show non-additive effects between certain parameters, notably those for which the incertitude is greatest.

Globally the model responds correctly to the various feeding situations used by Archimède (1992). However, some systematic biases were observed and shown by the breakdown of the error. The model was built for the rumen of a dairy cow and includes parameters from various sources, such as entry, exit and initial size parameters, which are those for a cow. However, more mechanistic parameters have been determined principally in sheep at maintenance and even *in vitro*. In addition, the main part of the data used in the validations comes from only 1 experimental source and this could be out of line with the averages seen in the literature (Archimède *et al*, 1994a, b).

The model responds correctly to variations in the level of ingestion and to a lesser extent the quantity of concentrate ingested (Robinson *et al*, 1986). This positive point is new compared to previous models. An important limit of the model is that it does not take into account nitrogen as a potential limiting factor for microbial proliferation. This choice was made because of the incertitude that exists in the variations of the quantity of nitrogen recycled depending on the diet. This inaccuracy does not limit functioning of the model with diets limited in energy, which is often the case in practice.

The constructed model allows a globally satisfactory prediction for duodenal fluxes for lysine and methionine compared with published measured values. The model can integrate without major modification of other amino acids in addition to lysine, methionine and branched-chain amino acids. This extension has already been made by the Cornell model (O'Connor *et al*, 1993). The satisfactory estimation of the lysine and methionine fluxes is in part due to an accurate estimation of microbial growth efficiency, which is close to that proposed by Ramangasoavina and Sauvant (1993). However, these authors using a larger number of data have shown that the PDI (INRA, 1988) and Cornell systems and the model of Baldwin *et al* (1987) frequently produced a large underestimation of the duodenal microbial protein flux. The values for LysDi and MetDi of the PDIE obtained by the model are quite consistent with values recently published in tables of raw materials (Rulquin *et al*, 1993). However, it is necessary to indicate that for these calculations the simulations are made in non-physiological conditions since the ration is made up of only 1 raw material, which may even be a concentrate. The robustness of the model allows the rapid estimation of LysDi and MetDi values for raw materials. In addition, the model can integrate the influence of 'mixed ration' factors, linked for example to the level of ingestion or the proportion of concentrate, a possibility which is not proposed by the actual LysDi and MetDi system.

The use of numeric integration methods did not pose a problem because the model is made up of equations which are mainly linear and if not very nearly linear. However, some parameters are chosen from of a large range of variations and can cause a bias in the results. Some of the parameters concerning the composition of feedstuffs can be analysed for sensitivity. Variations linearly linked to the composition (not shown) have been seen. The fact that few interac-

tions between factors are seen could be due to the structure of the mathematical model. This type of model generally produces stable solutions (Duflo, 1992). Numerous hypotheses underpin the construction of the model namely in the 'microorganism' section. Sensitivity analysis shows, owing to complete experiments (Kobilinsky and Monod, 1991), a large variation in the results through principle effects as well as first-order interactions. Up until now sensitivity analyses performed on the published models were used to study each individual parameter. The interactions between factors were ignored while they could influence the results as is the case for the model of Baldwin *et al* (1987).

CONCLUSION

The model presented has reused some now classic aspects of rumen digestion modelisation. This particularly concerns the definition of the substrates degraded by the microbes. However, many original aspects have been added compared to previously published models. The biological aspects it deals with concern namely the integration of variations in transit in relation to the diet ingested, the definition of the rumen fermentation profile from average results in the literature. The variations in the direct capture of molecules by the microorganisms was also taken into account together with the amino-acid composition of feedstuffs. For the last aspect, validation performed on published duodenal fluxes was particularly encouraging. The model is also original because of the procedure of internal validation. Finally, this model should be considered as the first step in the development of future versions which will be more mechanistic and which will integrate new characteristics of rumen metabolism (fats, other amino acids, minerals *etc*). A text (in the Dynamo language) of the model presented is available to readers on request.

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