

Comparison and evaluation of mechanistic rumen models

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

Rumen: Models: Lactation

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

Mathematical modelling is an important instrument for understanding and integrating knowledge (Baldwin, 1995). Detailed dynamic models that describe the mechanism of rumen function in cattle have been developed by Baldwin *et al.* (1987; BA), Danfær (1990; DA) and Dijkstra *et al.* (1992; DY). Although the BA and DA models are whole-animal models, in contrast to the DY model, the part that describes rumen function can easily be extracted in both cases because it is formulated as a separate module. Although the models have differing objectives and consequently apply different concepts and theories, all three models basically simulate the digestion, absorption and outflow of nutrients from the rumen. The model inputs required include daily feed intake, the composition and other characteristics of the feed, and several parameters describing rumen conditions. The BA model describes a mechanism to explain the rate at which nutrients become available for

the micro-organisms by including a representation of particle dynamics and the physical relationships between particulate substrate and micro-organisms. The DA model describes several pools for the constituents of the single type of micro-organism represented, whereas the DY model is concerned with studying the interactions between distinct microbial groups and with a more detailed description of fermentation processes. Extensive evaluations, by comparing observed with predicted values, have been published for the DY model (Neal *et al.* 1992).

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

MATERIALS AND METHODS

Selected data for evaluation

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

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

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

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

Diet...	1	2	3	4	5	6	7	Model†		
Chemical composition of diet (g/kg DM)										
SC	146	104	190	164	174	146	193	BA	DA	DY
DST*	6	121	18	4	3	4	3	BA	DA	DY
SST*‡	2	36	5	1	1	1	1	BA	DA	DY
NDF	409	351	407	373	397	406	333	BA	DA	DY
DNDF*	373	326	376	345	360	364	311	...	DA	DY
Lignin	12	10	13	13	14	27	12	BA
N	28.9	27.1	25.8	33.6	27.9	27.1	31.3	BA	DA	DY
SN*	5.6	8.5	6.5	16.8	12.4	10.1	14.0	BA	...	DY
UN*	1.1	1.2	1.4	1.1	1.4	1.6	1.1	...	DA	DY
NH ₃	1.4	2.1	1.6	4.2	3.1	2.5	3.5	BA	DA	DY
Lipid	39	41	31	37	37	41	37	BA	DA	DY
Parameter inputs§										
Intake (kg/d)	16.2	16.3	16.5	13.3	16.8	13.0	15.2	BA	DA	DY
<i>kd</i> _{DNDF} (/d)*	1.25	1.56	1.65	1.41	1.24	1.10	1.64	DY
<i>kd</i> _{DP} (/d)*	1.97	2.08	1.87	2.12	2.17	1.53	2.14	DY
<i>kd</i> _{DST} (/d)*	3.00	2.71	3.00	3.00	3.00	3.00	3.00	DY
<i>k</i> _{PLIQ} (/d)	3.72	3.89	3.89	4.51	4.13	2.86	2.26	BA	DA	DY
<i>k</i> _{OM} (/d)	0.77	1.22	0.94	0.79	0.82	0.58	0.58	BA	DA	DY
pH	6.14	6.16	6.15	6.63	6.12	6.20	6.13	BA	...	DY
pH-min	5.78	5.87	5.86	6.40	5.90	6.00	5.95	DY
Tf63 (h)	17.0	14.0	15.5	0.0	20.0	15.0	18.0	DY
Tf60 (h)	5.0	4.5	4.5	0.0	4.0	2.0	4.0	DY
Vol (litres)	64.5	53.8	57.7	54.6	64.0	67.8	72.9	BA	DA	DY

SC, soluble carbohydrate; DST, insoluble, degradable starch; SST, soluble starch; NDF, neutral-detergent fibre; DNDF, degradable NDF; SN, soluble nitrogen; UN, undegradable N; *k*_{PLIQ}, fractional liquid passage rate; *k*_{OM}, fractional organic matter passage rate; *kd*_{DNDF}, degradation rate NDF; *kd*_{DP}, degradation rate protein; *kd*_{DST}, degradation rate starch; pH, mean pH; pH-min, minimum pH; Tf63, time interval pH < 6.3; Tf60, time interval pH < 6.0; Vol, rumen non-dry matter.

* Value estimated from nylon bag incubations.

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

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

§ All remaining parameter inputs are described on pp. 566–567.

assumptions were applied to total diets. Rumen pool sizes and duodenal flows of microbial N (MN) were estimated with diaminopimelic acid (DAPA) as microbial marker (DAPA method). The microbial fraction in the duodenal flow of AAN was estimated with the observed fraction of MN in duodenal flow of non-NH₃ N (NAN).

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

Rumen models

The BA, DA and DY models were written in the Continuous System Modeling Program (CSMP) (Speckhart & Green, 1976). Simulations were performed with a fourth-order variable step length Runge-Kutta as integration method and with integration periods for the BA, DA and DY models of 20, 50 and 20 d respectively, which proved to be sufficient to reach a stable steady-state solution in every condition. Models were evaluated and compared on their steady-state solution. Certain parameter inputs for the BA and DA models were not measured, although their value clearly depends on the diet fed.

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

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

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

Experimental estimates of the comminution rates of large particles range from 0.96 to 3.12/d (Woodford & Murphy, 1988). Although techniques used in several studies with lactating dairy cows were different, reported estimates affirm such a large range (McLeod & Minson, 1988; Woodford & Murphy, 1988; Bosch, 1991). Sensitivity analysis (Baldwin *et al.* 1987) demonstrated that, in particular, inappropriately low estimates of fractional comminution rates can impair model prediction, although effects remain smaller with high-quality diets. Considering the low fibre content and the very high rumen digestibility of the

diets (91.8 (SD 1.2) % neutral-detergent fibre (NDF) degradability *in sacco* and 75.8 (SD 5.4) % rumen NDF digestion *in vivo*; Van Vuuren *et al.* 1992, 1993), the fractional comminution rate was increased from 4.5/d to 8.5/d. The resulting comminution rate in the model ($8.5/d \times 0.333 = 2.84/d$) approaches the highest estimates reported in the literature.

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

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

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

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

Model predictions

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

RESULTS

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

Rumen transactions of substrates

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

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

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

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

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

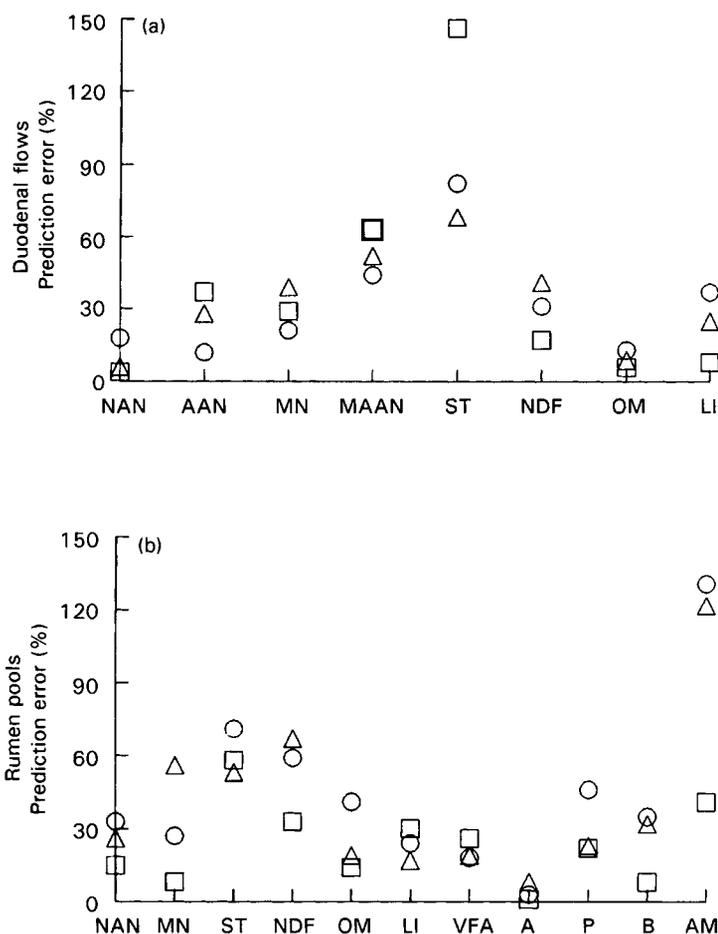


Fig. 1. Prediction errors of (a) duodenal flows and (b) rumen pools on seven test diets by the models of Baldwin (1995; \square), Danfær (1990; \circ) and Dijkstra *et al.* (1992; \triangle). Errors were calculated as square root of mean squared differences between predictions and observations, expressed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

Rumen transactions of nitrogen. The DA model predicted the highest degradation of feed AAN. Soluble and degraded AAN is either incorporated into microbial mass, fermented into VFA and NH_3 or escapes from the rumen. Differences amongst models in fermented AAN were opposite to differences in incorporated feed AAN (on average there was 64, 50 and 56 % fermentation, and 14, 35 and 23 % incorporation of the rumen input of feed AAN with the BA, DA and DY models respectively; Table 3). Considerable differences remained amongst models in the predicted escape of feed N to the duodenum.

Duodenal flows of nitrogen. The BA and DY models predicted the duodenal flow of NAN accurately, whereas the DA model underpredicted (Figs. 1(a) and 4(a)). However, the microbial contribution to duodenal NAN appeared to be overpredicted in all three models (Figs. 1(a) and 4(c)).

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

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

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

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

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

Assuming feed non-AA NAN is input to the rumen NH₃ pool, predicted duodenal flows of protein were considered to consist of AAN only. Duodenal flow of NAN minus MN and duodenal flow of AAN minus MAAN provide estimates of feed N and feed AAN respectively, that escapes from the rumen. Comparison of these calculations on experimental data and model predictions (Table 3) shows that the duodenal flow of non-AA NAN (e.g. nucleic acids) is strongly underpredicted by all models. On average, the BA, DA and DY models predicted 81, 57 and 80 % of observed duodenal flow of feed N, 136, 93 and 132 % of feed AAN, and 30, 38 and 65 % of non-AA NAN respectively (prediction errors of feed N 18, 47 and 25 %, of feed AAN 38, 24 and 27 %, and of non-AA NAN 71, 62 and 36 % with the BA, DA and DY models respectively; Table 3).

Products of microbial substrate utilization

Fermentation end-products. Because VFA production was not measured, models could only be evaluated on the concentration and molar percentage of VFA. Differences between models in the predicted production of VFA were inverted in the predicted concentrations because of different absorption rates. Although the BA model predicted the largest VFA production, it underpredicted rumen concentration of VFA (Fig. 5(a)) with the largest

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

Diet ...	1	2	3	4	5	6	7
	Duodenal flows (g N/d)						
Feed N*							
Exp	154	164	154	87	116	72	85
BA	126	150	125	75	90	54	61
DA	61	66	60	67	76	51	58
DY	92	128	95	94	92	63	73
Microbial N							
Exp	255	260	242	220	255	190	224
BA	264	247	277	236	282	212	240
DA	271	274	278	158	274	179	201
DY	315	295	323	247	315	222	242
Feed AAN*							
Exp	80	97	78	55	90	36	68
BA	127	150	123	76	91	55	60
DA	61	67	60	67	75	51	58
DY	92	129	94	94	92	63	74
Microbial AAN							
Exp	172	176	163	152	171	124	156
BA	226	212	238	202	242	181	206
DA	217	219	223	127	220	143	162
DY	227	212	233	178	227	160	174
Non-AAN NAN							
Exp	157	151	155	100	110	102	85
BA	37	35	41	33	39	30	35
DA	54	54	55	31	55	36	39
DY	88	82	91	69	88	62	67
	Transactions (g N/d)						
Degraded AAN							
BA	253	156	198	152	173	168	204
DA	414	372	371	357	374	299	410
DY	337	244	271	196	222	187	232
Fermented AAN							
BA	267	202	215	272	268	230	331
DA	183	191	190	198	193	185	245
DY	248	184	204	214	221	203	304
Incorporated AAN†							
BA	60	64	70	51	67	42	38
DA	204	153	152	129	152	86	124
DY	114	107	110	91	112	60	52
Net MN synthesis from NH ₃ ‡							
BA	204	183	207	185	215	170	202
DA	67	121	126	29	122	93	77
DY	201	188	213	156	203	162	190

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

* Feed AAN = total AAN - microbial AAN; feed N = NAN - microbial N.

† Input AAN - duodenal flow of feed AAN - fermented AAN.

‡ Duodenal flow of MN - incorporated AAN.

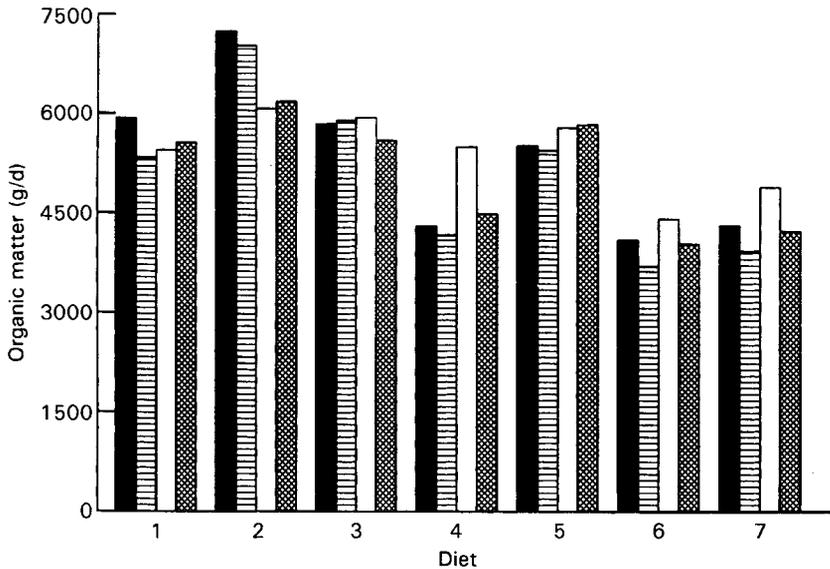


Fig. 2. Comparison of observed (■) and predicted values of duodenal flow of organic matter on seven test diets by the models of Baldwin (1995; ▨), Danfær (1990; □) and Dijkstra *et al.* (1992; ▩).

prediction error (Fig. 1(b)). Model predictions of the molar percentage of individual VFA deviated strongly from the observed percentages (Figs. 1(b) and 6(a, b and c)). Only the DY model includes the formation of valerate (overpredicted with a prediction error of 515 %, results not shown). This extra VFA influences VFA predictions, because attribution of the C in valerate to acetate, propionate, or butyrate would increase the VFA production and alter the molar percentages. NH_3 is an end-product of protein fermentation in addition to VFA and predicted and observed rumen concentrations of NH_3 differed strongly (Figs. 1(b) and 5(b)). In particular the response of the BA and DY models (except on diet 7 with the DY model) to input variation corresponded to the observed response in concentration of NH_3 .

Microbial synthesis. The observed duodenal flows of MN and MAAN on diets 4–7 were much lower with the AA profile method than with the DAPA method (Fig. 4, (c) and (d)). Because results with the DAPA method were considered to be more accurate, for reasons that will be discussed, model predictions were evaluated only on observations with the DAPA method.

The models predicted similar duodenal flows of MN (Fig. 4, (c) and (d)). Consequently, differences amongst models in the predicted incorporation of AAN (26, 77 and 45 % of duodenal flow of MN with the BA, DA and DY models respectively) compensated with opposite differences in net MN synthesis from NH_3 (78, 36 and 58 % of duodenal flow of MN respectively). In contrast to the DY model, the assumed AAN content in MN seemed to be too high in the BA and DA models because duodenal flow of MAAN was more overpredicted than that of MN (Fig. 4, (c) and (d)). Further, absolute overpredictions of the duodenal flow of MAAN and AAN were of a similar size in the BA and DY models (Fig. 4, (b) and (d)). This is illustrated by average prediction errors of the duodenal flow of MAAN v. AAN in the BA, DA and DY models of 40 v. 37, 27 v. 12, and 31 v. 28 % respectively (Fig. 1(a)).

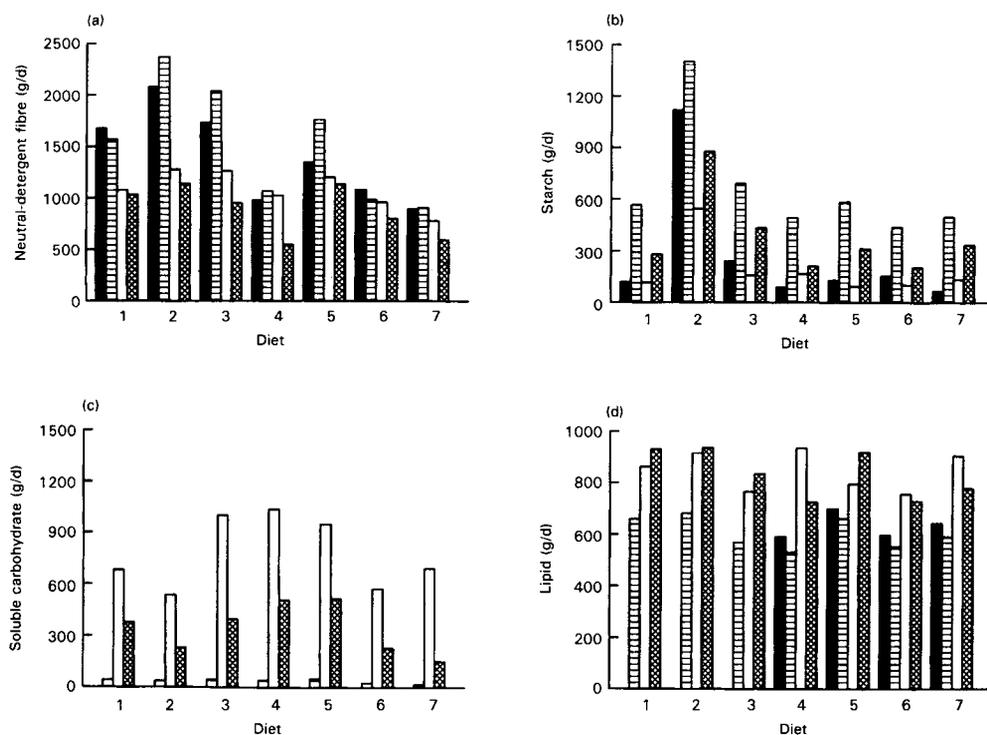


Fig. 3. Comparison of observed (■) and predicted values of the duodenal flow of (a) neutral-detergent fibre, (b) starch, including solubilized starch, (c) soluble carbohydrate including solubilized NDF and excluding solubilized starch and (d) lipids on seven test diets by the models of Baldwin (1995; ▨), Danfær (1990; □) and Dijkstra *et al.* (1992; ⊠).

DISCUSSION

Input parameters

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

Choosing parameter values that seem more appropriate than the published estimates caused the BA model to predict duodenal flow of NDF more accurately than the other

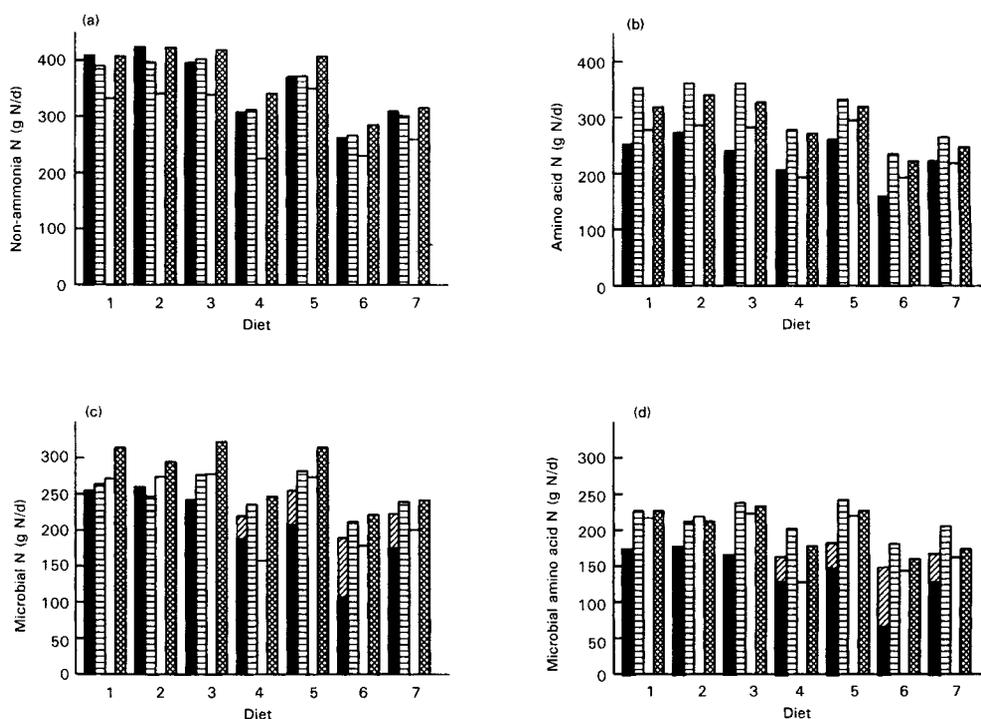


Fig. 4. Comparison of observed (■) and predicted values of the duodenal flow of (a) non-ammonia N, (b) amino acid N, (c) microbial N and (d) microbial amino acid N on seven test diets by the models of Baldwin (1995; ▨), Danfær (1990; ▤) and Dijkstra *et al.* (1992; ▩). Observed microbial N fractions obtained with the amino acid profile method (■) in diets 4–7 and with the diaminopimelic acid (DAPA) method (▨) in diets 1–3.

models. However, other parameter estimates for rumen outflow might have improved NDF prediction in the DA and DY models also.

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

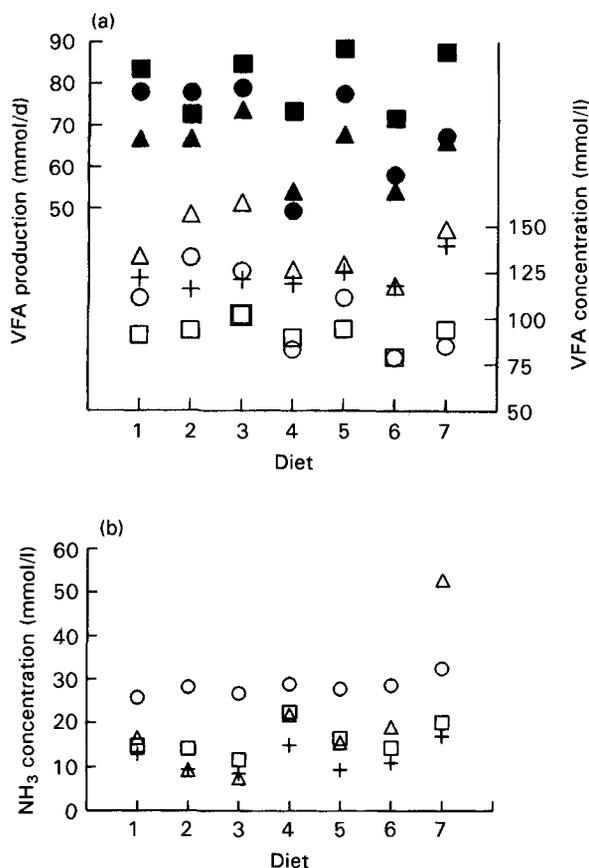


Fig. 5. Comparison of observed (+) and predicted values of (a) volatile fatty acid (VFA) production (■, ●, ▲) and concentration (□, ○, △) and (b) ammonia concentration on seven test diets by the models of Baldwin (1995; □), Danfær (1990; ○) and Dijkstra *et al.* (1992; △).

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

Nutrient flows

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

With the assumption that all feed non-AA NAN is immediately hydrolysed and constitutes an input to the rumen NH₃ pool, the predicted duodenal flow of non-AA NAN is assumed to be entirely of microbial origin and escaped feed N to be 100% AAN.

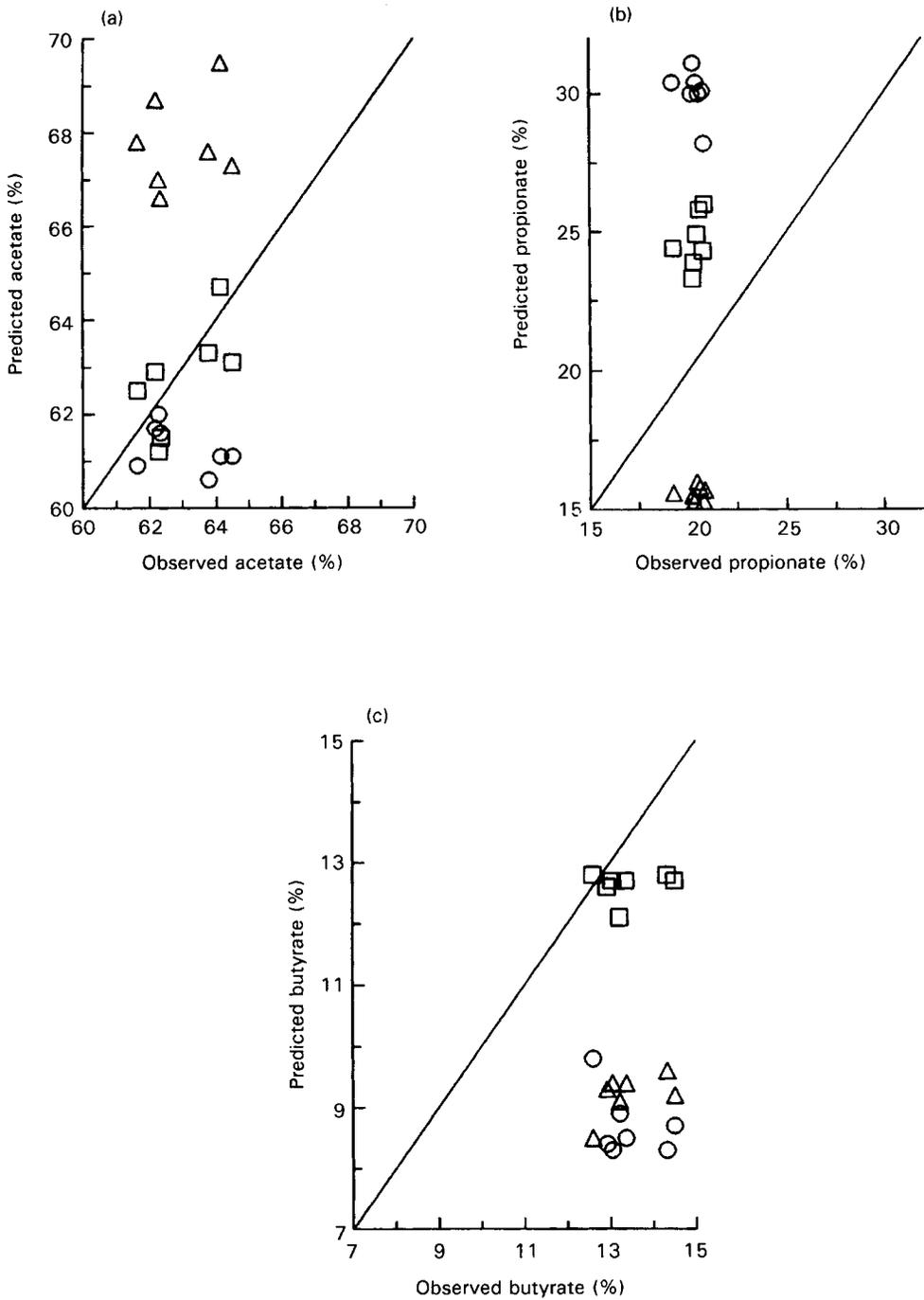


Fig. 6. Comparison of observed and predicted values of (a) molar percentage of acetate, (b) molar percentage of propionate and (c) molar percentage of butyrate on seven test diets by the models of Baldwin (1995; □), Danfær (1990; ○) and Dijkstra *et al.* (1992; △). In the model of Dijkstra *et al.* (1992),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

However, the observed duodenal flows of non-AA NAN were much higher than the predicted values (Table 3), suggesting that some feed non-AA NAN did escape from the rumen. Considering rumen protein as crude protein ($\text{g N} \times 6.25$) instead of true protein (no NH_3 input from soluble N) will reduce predicted error of the duodenal flow of AAN and non-AA NAN. However, caution is necessary with explaining inaccurate prediction from the model representations because also the experimental estimates may be seriously biased.

Of all the measured duodenal flows, that of microbial matter probably has the largest standard deviation and is the least reliable. Therefore, not only the accuracy of model predictions but also the reliability of observed MN in particular should be evaluated. With the AA profile method, estimates of MAAN or MN were clearly lower with the AA profile method than with the DAPA method (Fig. 4, (c) and (d)). The percentage N of microbial origin was used to estimate the duodenal flow of MAAN and MN from the observed duodenal flow of AAN and NAN respectively, which seems to be valid at least on diets 4–7 because it resulted in ratios of duodenal flow of MAAN and MN that were comparable with that of the independently observed amounts of AAN and total N found in microbial matter isolated from duodenal samples (68 % AAN). Variability of prediction errors was larger with the AA profile method and estimates of duodenal flow of MN and MAAN on diet 6 are so low that they are highly unlikely. The larger deviations between the predicted and observed duodenal flows of MN and MAAN with the AA profile method does not agree with the uniform prediction error of the duodenal flow of NAN or AAN for all diets (Fig. 4, (a) and (b)). From these arguments it may be concluded that the AA profile method in particular resulted in severely biased and inconsistent estimates of microbial mass in samples of the duodenal contents. In a direct comparison of alternative methods for the quantification of microbial matter, Siddons *et al.* (1982) also established much higher estimates of microbial N in rumen digesta of sheep with the DAPA method compared with the AA profile method. In contrast, Voigt *et al.* (1991) established low but comparable estimates with the AA profile and DAPA method in duodenal digesta of dairy cows. Thus, highly variable results have been obtained with different methods as well as within a single method of microbial quantification.

Further, protozoa were excluded with the isolation of microbial matter from digesta samples and also the DAPA method largely excludes protozoa (Broderick & Merchen, 1992). Thus, the presence of substantial amounts of protozoal N in duodenal MN might result in an underestimated experimental value of MN and consequently an overestimated duodenal flow of feed N, feed AAN and non-AAN NAN. A more substantial contribution of protozoal N could be expected with diets 2 and 3 containing 330 g concentrates/kg DM intake (Dijkstra, 1994); however, there is no indication of a larger prediction error of MN flow (Fig. 4(c)). An indication of an underestimated duodenal MN flow might be the much higher duodenal flows of non-AA NAN than can be explained by the outflow of microbial non-AA NAN (Table 3). In conclusion, there is a strong possibility of large bias in the experimental estimates of duodenal flow of MN and MAAN which seriously limits their use for model evaluation.

The models predicted different amounts of fermented substrate (Tables 2 and 3), accompanied by different VFA productions (Fig. 5(a)). For predicting nutrient flows, the production of individual VFA is of particular interest because they are the largest energy fraction absorbed from the gastrointestinal tract (Reynolds *et al.* 1994). However, no such observations were made in the experiments used. The only VFA observations available were those of concentrations in rumen fluid. The DY model uses the estimated VFA coefficients from Murphy *et al.* (1982) which describe the stoichiometry of the production

of acetic, propionic, butyric and valeric acids with fermentation of soluble carbohydrate, starch, hemicellulose, cellulose and protein. These coefficient values resulted in a consistent underprediction of the molar percentages of acetic and butyric acids, and overprediction of those of propionic and valeric acids in rumen fluid. The BA model also uses these coefficient estimates, but with 1.0 mol propionic acid and 0.5 mol butyric acid substituted for 1.0 mol valeric acid. Further, coefficient estimates for protein fermentation were altered and VFA coefficients with fermentation of soluble carbohydrate and starch were made dependent on rumen pH (Argyle & Baldwin, 1988). These updated coefficient estimates resulted in the most accurate predictions of the molar percentages of individual VFA in rumen fluid. Earlier coefficient estimates of Baldwin *et al.* (1970) were applied in the DA model and resulted in the worst predictions. The different VFA absorption kinetics in the BA, DA and DY models will have contributed to these different results. The deviations found between predicted and observed molar percentages of individual VFA in rumen fluid are to be explained by incorrect prediction of the amount of fermented substrate, by incorrect coefficient values, or by incorrect absorption kinetics. Yet, it is uncertain to what extent these deviations may be considered representative of rumen VFA production.

Differences amongst models in the assumed microbial composition and substrate requirements for microbial synthesis can partially originate from the assumption in the BA and DA models that microbes incorporate rumen lipid, whereas the DY model ignores this. Besides the synthesis of the protein fraction in microbial mass, that of the lipid fraction might require the highest amount of substrate for generation of ATP or for incorporation into microbial mass (Czerkawski, 1986; Dijkstra *et al.* 1992; Baldwin, 1995). The assumptions made in the BA, DA and DY models on the utilization of feed lipid will have influenced the parametrizations adopted for substrate requirement with microbial synthesis. Notwithstanding the small lipid content of the diets in the present study (31 to 41 g lipid/kg DM intake; Van Vuuren *et al.* 1992, 1993), such considerations are necessary to explain the higher carbohydrate incorporation in the DY model. Thus, different predictions of the amounts of carbohydrate, AAN and NH₃ that are incorporated and fermented with microbial synthesis must be explained from a different origin of the microbiological data used with model construction or from different assumptions on rumen conditions for microbial growth (Russell, 1984; Van Gylswyk & Schwartz, 1984; Czerkawski, 1986). To conclude this section on predicted nutrient flows, considerable differences exist in the predicted rumen transactions of carbohydrates and N. Although the differences between the BA, DA and DY models largely compensated for each other and resulted in more-or-less comparable predictions of the duodenal flows of OM, total carbohydrate, NAN and MN, large differences were established in the prediction of rumen transactions, duodenal flows of the individual carbohydrate and N fractions, and production rates of individual VFA.

Implications for future research

None of the models appeared to be clearly superior in predicting the nutrient flows to the duodenum. Calibration of the BA, DA and DY models apparently made them all predict the generally observed duodenal flows of OM, NAN and MN reasonably well. These comparable predictions result from a compensation of large and systematic differences that occurred in the simulated intraruminal flows. Forcing similar entry rates of soluble

substrates into rumen fluid (according to the model descriptions the only substrate that can be utilized by micro-organisms) in the BA, DA and DY models was demonstrated to result in comparable differences in microbial metabolism to those found in the present study (Bannink & de Visser, 1995). Therefore, next to a different formulation of substrate availability, a different formulation of the microbial metabolism seems to cause the model differences established. Broadening the range of experimental conditions concerning amounts of feed intake, roughage:concentrate ratios, N content in feed, and types of roughage and concentrate will probably result in more distinct model behaviour (Dijkstra *et al.* 1992; Neal *et al.* 1992) and enable a more thorough evaluation of the models.

One aspect of rumen modelling that requires further research is resolving the representation of the microbial metabolism. The models tested differ strongly in concepts and theories applied because of the different objectives they were built with. The validity of these representations could not be tested with the observations used in the present study, although they appear to be the main distinction between the BA, DA and DY models and were shown to result in differing profiles of nutrient flows. More precise and detailed measurement of microbial and non-utilized feed fractions in carbohydrate, nitrogenous and other constituents in duodenal OM, might identify which representation of microbial metabolism is able to describe the transactions of substrates in the rumen. In this respect, the representation of microbial metabolism also needs to address the variation in the composition of microbial matter with changing dietary conditions (Danfær, 1990; Dijkstra *et al.* 1992). Further, most ingested OM is fermented into VFA, but VFA production cannot be evaluated with observations of rumen concentrations. Rumen concentration of NH_3 additionally concerns MN synthesis from NH_3 and recycling of NH_3 to the rumen. The inconsistency between models in the present study indicates that further research is needed to represent these processes appropriately in rumen models.

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

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

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