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Design and evaluation of a non-steady state rumen model

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Abstract

A dynamic simulation model of digestion and absorption of nutrients was modified and evaluated under non-steady state conditions. The results of detailed grazing experiments, including allowed grazing times (Exp. 1), combinations of rumen fill and starvation length before grazing (Exp. 2) and contrasting sward masses and sward heights (Exp. 3) as main treatments were used as reference values. The model was modified to run under a discontinuous feed input of ryegrass. Neutral detergent fibre (NDF) and nitrogen rumen pools were predicted with a relatively low root mean square prediction error (MSPE) of the observed means (11%) in Exps. 1 and 2, but a higher value (18%) was observed in Exp. 3. This significantly higher root MSPE was ascribed to the long period (up to 20.5 hours) of starvation that followed grazing. Prediction was poorer for organic matter rumen pool (root MSPE of 16%) than for NDF and N rumen pools, which requires further validation. Volatile fatty acid (VFA) rumen pool and VFA concentration were predicted with a root MSPE of the observed mean of 32–33%, which was close to the random variation observed in the experiments. Ammonia rumen pool was poorly predicted and the way ammonia is represented in the model must be modified to predict ammonia production and absorption under non-steady state conditions. The model can be used to predict ruminal digestion and absorption of nutrients (except ammonia) of grazing lactating dairy cows under discontinuous feeding regimens provided large periods of starvation are avoided.

Key words: dairy cows, rumen model, non-steady state, grazing, digestion, *Lolium perenne*.

Introduction

Dry matter intake (DMI) is the dominant process for assessing animal productivity (Forbes, 1995). In forage-fed animals, the processes that occur in the rumen play a determinant role in the amount and type of nutrients absorbed (Tamminga & Van Vuuren, 1996) and in the control of DMI (Grovum, 1995).

The progress made in the representation and quantification of the rumen fermen-

tation process in models simulating whole rumen function has been significant, but important gaps in knowledge and representation still remain (e.g. Bannink & De Visser, 1997; Dijkstra, 1994). Recently an extensive evaluation of whole rumen function models has been made (Dijkstra & France, 1996) and a number of issues that require further research were addressed. One of these issues is the need for models capable of representing discontinuous feeding regimes in combination with rumen pools that vary in size with time.

For the representation and prediction of the unique processes of ingestion and digestion under grazing, a rumen model capable of simulating fermentation processes in discontinuous feeding regimens is essential. The present paper describes the modification of a mechanistic dynamic model aimed at predicting digestion and absorption of nutrients in cattle fed sugarcane-based diets under steady state conditions (Dijkstra *et al.*, 1996) and the evaluation of this model for grass-based diets under non-steady state conditions. For this evaluation the results were used of grazing experiments with allowed grazing times, combinations of rumen fill and starvation length before grazing, and contrasting sward masses and sward heights as main treatments.

Material and methods

A dynamic mechanistic model developed by Dijkstra *et al.* (1996) to predict digestion and absorption of nutrients in cattle fed sugarcane-based diets under steady state conditions was modified to simulate a discontinuous feeding regimen of ryegrass (*Lolium perenne*). Three grazing experiments (Chilibroste *et al.*, 1997, 1998a, 2000) were used for the evaluation of this modified model. The experiments yielded a large data set (n = 104 individual observations) obtained with lactating dairy cows grazing on ryegrass pasture. Most of the inputs required by the model were recorded during the experiments. The remainder was obtained as explained in the section *Model inputs.*

Description of the model

The rumen model comprises 11 state variables representing four carbohydrate fractions, four nitrogen (N) fractions, two fatty acid fractions and one microbial rumen pool. The four carbohydrate fractions included undegradable neutral detergent fibre (NDF), degradable NDF, insoluble starch, and soluble starch and sugars. The four N fractions included undegradable protein, insoluble but degradable protein, soluble protein, and ammonia. The two fatty acid fractions included long-chain fatty acids and volatile fatty acids (VFA). All pools were expressed in grammes except the VFA pools, which were expressed in moles. The rate of change of each pool with time was described with a single differential equation integrating inflow, outflow, synthesis and utilization of each fraction. The flux equations were described by Michaelis-Menten and mass-action formula. For numerical integration, a fourth-order Runge-Kutta method was used, which was run for a number of days to achieve steady state

solutions. Details about model parameterization and the flux equations that constitute the model have been described in detail by Dijkstra *et al.* (1996).

Description of the experimental protocols

Detailed descriptions of the experimental protocols have been published elsewhere (Chilibroste *et al.*, 1997, 1998a, 2000). The general procedure for an experimental day is shown in Figure 1. After morning milking, rumen evacuation was carried out. After each evacuation, the cows were placed in their respective grazing plots until the allowed grazing time was over (Exp. 1) or until the cows voluntarily stopped grazing (Exps. 2 and 3). Immediately after grazing, each cow was transferred to the barn where its rumen was evacuated again. After the replacement of this second rumen evacuation, the cows were starved till the evening (Exp. 1 and 2) or till next morning (Exp. 3), when a third rumen evacuation was carried out (Figure 1).

Model modifications

To be able to run the model under non-steady state conditions the following modifications were introduced:

1. *Time*. The original model was developed to run with hour as unit of time. Since the grazing observations were done at 10-minute intervals, the unit of time in the model is minute. The rumen pool sizes measured by evacuation before grazing started (evacuation 1, Figure 1) were used as initial values at time 0 and the model was run until the end of the starvation period (evacuation 3, Figure 1). All time-dependent parameters (production, absorption, synthesis, utilization and fractional degradation or passage rates) were converted to operate on a minute basis.
2. *Dry matter intake* (DMI). Instead of assuming a steady state with a constant input of nutrients (Dijkstra *et al.*, 1996), a discontinuous feed input was programmed. Grazing time was defined as the time elapsed from the moment the cows were placed on the experimental plots until the moment they were transferred. During grazing, bite rate was recorded for each cow by the same observer at 10-minute intervals. The total number of bites was calculated as the product of grazing time

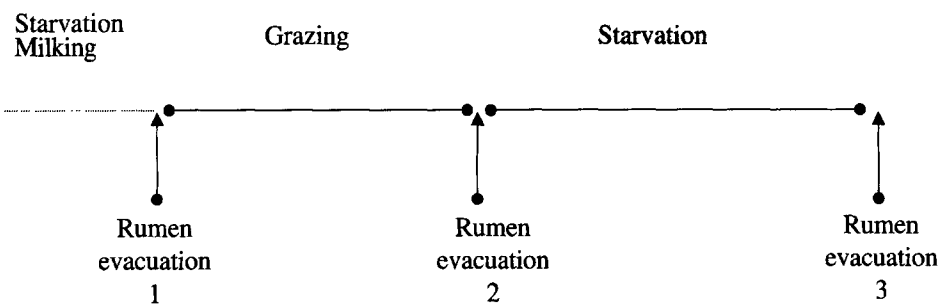

Figure 1. Diagrammatic representation of the experimental protocol.

and average bite rate. In the model, DMI at each integration step was calculated for each individual cow as the product of the observed bite rate and the estimated bite mass. For detailed methodological protocols of grazing observations see Chilibroste *et al.* (1997, 2000).

3. *Rumen pools*. Three new rumen pools were calculated from the rumen pools originally conceived in the model. Organic matter (OM) rumen pool was calculated as the sum of crude protein, carbohydrates and long-chain fatty acids microbial and non-microbial rumen pools. Dry matter (DM) rumen pool was calculated from OM rumen pool and from the ash fraction in the DM (114.8 ± 11.7 g per kg DM; n = 52). N rumen pool was calculated by the summation of the rumen pools of N in the feed (undegradable, degradable and soluble N), N in the ammonia pool and N in the microbial pool. It was assumed that the crude protein (CP) content of the microbial biomass was 650 g per kg DM (Dijkstra *et al.*, 1996).
4. *Rumen volume* (V). To be able to simulate under non-steady state conditions, V has to be considered a non-steady state variable instead of a constant dietary-specific variable like in the original model. A large variability has been observed in V when grasses and legumes of low DM content are fed to cattle (Waghorn, 1986; Chilibroste *et al.*, 1997; 2000). A non-linear relationship between DM rumen pool size (*DMRP*; kg) and DM percentage of rumen contents (*DMC*; %) as derived from the data set (Figure 2) was used to estimate rumen V from DM rumen pool at each integration step. This yielded the following equation:

$$DMC = 12.05\ (\pm 0.19) \times (1 - e^{-0.32\ (\pm 0.17) \times DMRP});\ \text{Relative SE} = 1.24.$$

5. *Microbial population*. In the original model – based on sugarcane-fed dairy cattle – it was assumed that protozoal and bacterial biomass in the rumen constitute 40 and 60%, respectively, of the total microbial biomass in the rumen. These percentages were changed to 20 and 80, respectively, to represent the situation in which

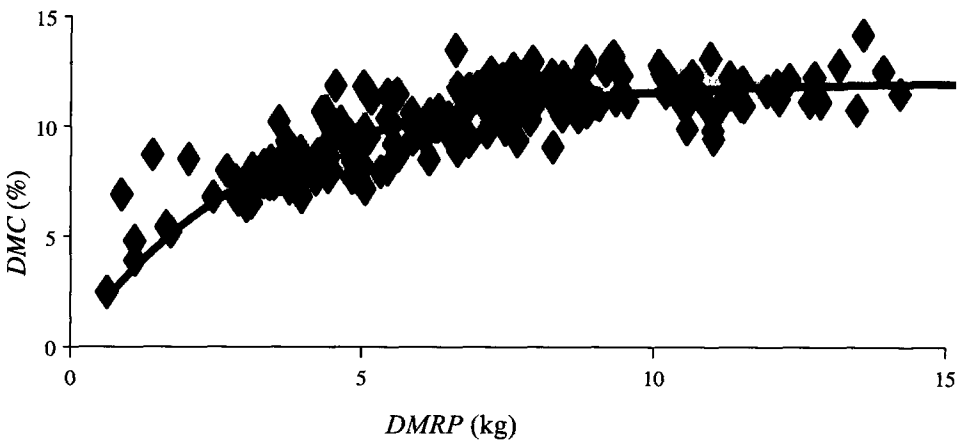

Figure 2. Predicted (line) versus observed (symbols) DM content (*DMC*) of the DM rumen pools (*DMRP*) for Experiments 1, 2 and 3.

cows are fed cell-wall-rich ryegrass diets (Hungate, 1966). Other assumptions about the microbial population, like the proportion of bacteria attached (75%) and not attached (25%) to the solid fraction, and the fractional outflow rates of protozoa and bacteria, were not modified.

Model inputs

Feeds

In Table 1 the feed inputs required by the model are summarized for each experiment. Exp. 3 comprised different days of sward regrowth as treatments, so three groups of input values were used. One set of input values was used for the first 16 days of regrowth, when little variation in sward chemical composition was observed, and two more sets of input values for the measurements on days 22 and 30, when changes in sward chemical composition had become evident (Chilibroste *et al.*, 2000).

The values presented in Table 1 were derived from the chemical composition of the grass (Chilibroste *et al.*, 1997, 2000). A constant chemical composition was assumed for each individual bite. The compositional characteristics undegradable (U), degradable (D) and soluble (S) of feed carbohydrates and CP for Exps. 1 and 2 were derived from Van Vuuren *et al.* (1992, 1993), and for Exp. 3 from Chilibroste *et al.* (1998b). It was assumed that ammonia, VFA and starch contents of the grass were 0. Furthermore, a constant lipid content of 60 g per kg DM in the grass was assumed. Passage rates of the solid and liquid fractions and degradation rates of degradable NDF and insoluble, degradable CP were assumed to be the same in the three experiments and were set at 3.5, 8.0, 4.0 and 8.0% per hour (0.058, 0.133, 0.066 and 0.133% per minute), respectively. These values were derived from Van Vuuren *et al.* (1992) and from our own nylon-bag incubations (P. Chilibroste, unpublished data).

Initial rumen conditions

Initial rumen pools were the rumen pools determined in the evacuation before grazing (evacuation 1, Figure 1). For the three experiments, 10% of the N was assumed to be soluble, 40% insoluble but potentially degradable, and 50% undegradable. Of

Table 1. Fractions (g per kg DM) undegradable (PU), degradable (PD) and soluble (PS) crude protein, neutral detergent fibre (NDF), degradable NDF (NDF Deg.) and water-soluble carbohydrates (CHO Sol.) in the grass eaten by the cows, in Experiments 1, 2 and 3.

Fraction	Exp. 1	Exp. 2	Exp. 3		
			Days 6–16	Day 22	Day 30
PU	8.5	17.1	17.1	21.7	11.8
PD	76.7	153.5	153.2	127.2	106.0
PS	45.9	91.9	91.7	76.1	63.4
NDF	473.5	511.1	430.1	466.9	531.9
NDF Deg.	378.8	409.2	344.2	373.5	425.5
CHO Sol.	242.2	100.2	150.2	150.0	150.0

the initial NDF rumen pool 50% was estimated to be undegradable in Exps. 1 and 2, and 40% in Exp. 3. These percentages were derived from a simulation exercise in which NDF and CP rumen pools observed before starvation (Chilibroste *et al.*, 1998a, 2000) were used as initial values, assuming a ratio of degradable : undegradable material as reported by Van Vuuren *et al.* (1992). During simulation the rumen pools were exposed to passage (undegradable fraction) and to passage plus degradation (degradable fraction) for the period that the cows were starved. At the end of the simulation period the ratio degradable : undegradable fractions was recalculated. The OM residues after 144 hours of incubation *in vitro* (Chilibroste *et al.*, 1999) – after correction for microbial contamination – also gave an indication of degradability of the NDF rumen pools, since NDF made the major contribution to the OM rumen pools (Chilibroste *et al.*, 1998a; 2000). Both approaches yielded similar figures. Initial pool size of rumen lipid was set at 60 g per kg DM, and soluble carbohydrates at 0.6 g l^{-1} (J. Dijkstra, personal communication).

Comparison of simulated and experimental values

The observed values of OM, NDF, N, VFA and ammonia rumen pools in the evacuations after grazing and after starvation (evacuations 2 and 3; Figure 1) were compared with the values predicted by the model. An assessment of the error of predicted relative to observed values was made by calculating the mean square prediction error (MSPE), using the following equation:

$$\text{MSPE} = \sum_{i=1}^{n}(O_i - P_i)^2/n$$

where
i = 1, 2, ...n,
n = number of observations, and
O_i and P_i are the observed and predicted values, respectively.

The MSPE was split into error due to the overall bias of prediction, error due to deviation of the regression slope from unity, and error due to the disturbance (random variation) (Bibby & Toutenburg, 1977).

Results and discussion

In Figure 3, the simulated OM, NDF and N rumen pools are plotted against the observed values. The predicted OM pool tended to be lower than the observed OM pool (root MSPE of 16% of observed mean, and overall bias and deviation of the regression slope from unity contributing 33 and 23%, respectively, to the MSPE). The root MSPE for the NDF rumen pool was also 16% of the observed mean, but without clear bias (80% of MSPE was attributed to random disturbance). The rumen N pool was predicted well, with a root MSPE of 12% of the observed mean and 80% of MSPE attributed to random disturbance. Because N and NDF pools were fitted well

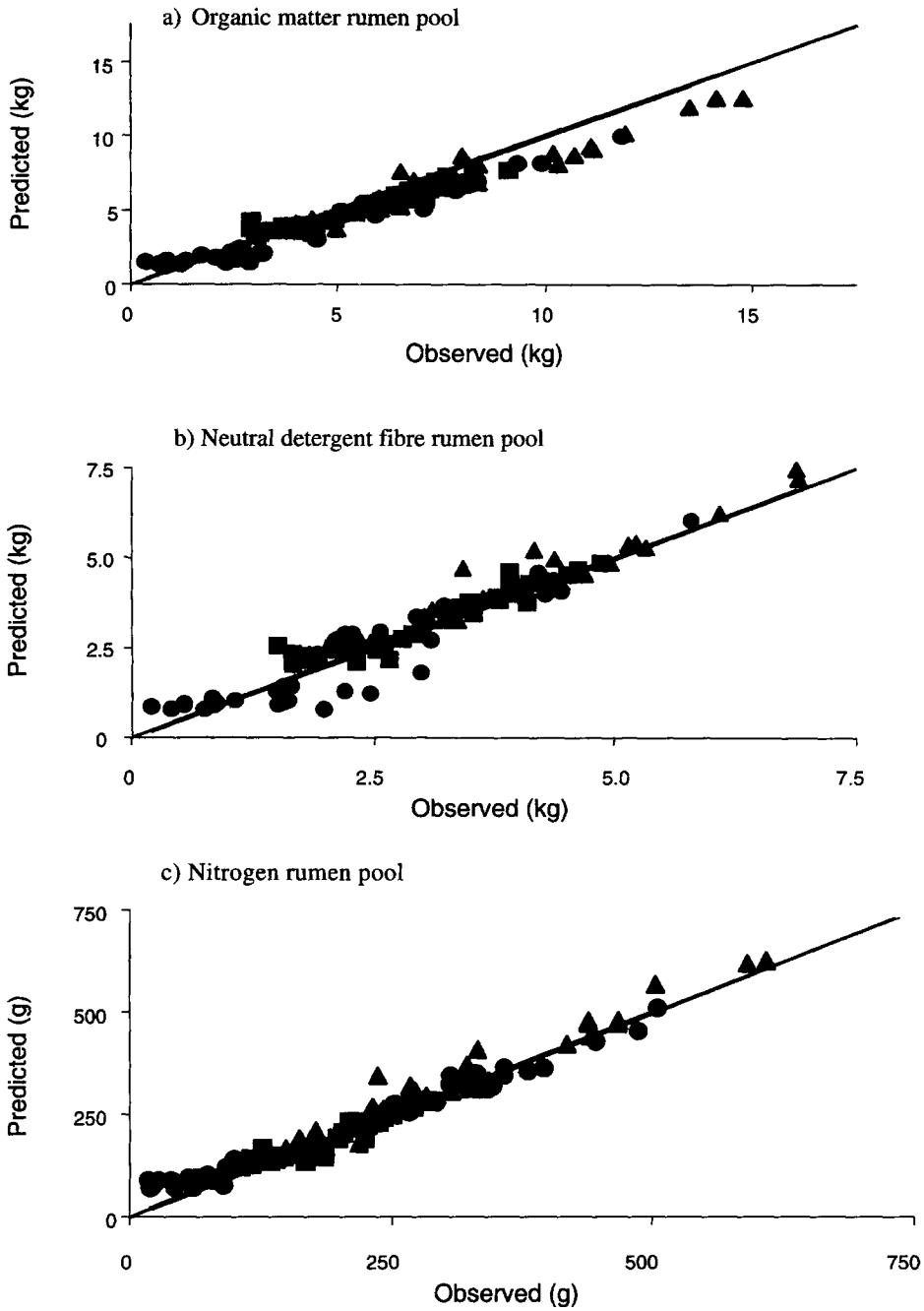

Figure 3. Predicted (line) versus observed (symbols) values for organic matter, neutral detergent fibre and nitrogen rumen pools. ■: Experiment 1; ▲: Experiment 2; ●: Experiment 3.

without obvious bias, the poorer fit of OM pool size must be ascribed to non-protein, non-NDF organic matter in the rumen, i.e., soluble sugars and crude fat. Since systematic differences between observed and predicted pools within experiments may exist that will not appear in the combined analysis, the root MSPE and the contribution of random variation to MSPE within experiments are presented in Table 2. For the three pools, the root MSPE of the observed mean was larger in Exp. 3 than in Exps. 1 and 2.

Few, if any, evaluations of simulation models are available that predict rumen pool sizes under non-steady state conditions. Neal *et al.* (1992) observed MSPE values of 19 and 25% for the prediction of rumen bacterial N pools under assumed steady state conditions at different levels of intake and different levels of dietary starch, respectively. Bannink *et al.* (1997) evaluated the rumen models of Baldwin *et al.* (1987), Danfær (1990) and Dijkstra *et al.* (1992) which were run under steady state conditions, using observations on cows fed grass-based diets. In general, they observed a much larger prediction error for OM, NDF and N rumen pool sizes than for corresponding duodenal flows. France *et al.* (1982) evaluated a sheep rumen model for continuous and discontinuous feed inputs and found good predictions of rumen outflow with continuous feed input, but large errors in outflow occurred with discontinuous input. The MSPE observed in our experiments for OM, NDF and N rumen pool sizes also compare favourably with predicted duodenal flows of NDF and N under assumed steady state conditions (Van Straalen, 1995; Neal *et al.*, 1992).

Either an underestimate of OM intake or an overestimate of OM clearance from the rumen could explain a predicted OM rumen pool lower than the observed value. Although discontinuous OM intake was an input during simulation, chemical composition during the grazing session was assumed constant (Table 1). An estimate of the selectivity in our experiments showed a high residual variability due to the low sward utilization in the experiments (Meijs, 1981), which is likely to be the main source of error in terms of feed inputs to the model.

OM clearance from the rumen is the result of two simultaneous processes: degradation and passage. The latter has major effects on model prediction (Dijkstra & France, 1996). In our experiments we used the clearance rate (k_{cl}) of acid detergent lignin (ADL) as an indicator of solid passage rate, assuming that no degradation of ADL occurs. The observed k_{cl} values of ADL (3.9 ± 1.7% per hour) were within the wide range of passage rates estimated by Owens & Goetsch (1986) and Sauvant *et*

Table 2. Root mean square prediction error (MSPE, % of observed mean) and the contribution of random variation to the MSPE (Random, % of MSPE) of the fractions organic matter (OM), neutral detergent fibre (NDF) and nitrogen (N) rumen pools, in Experiments 1, 2 and 3.

Fraction	Exp. 1		Exp. 2		Exp. 3	
	MSPE	Random	MSPE	Random	MSPE	Random
OM	13.1	49.1	14.3	37.4	19.7	40.2
NDF	11.9	75.4	11.7	46.8	21.5	89.6
N	8.4	99.7	11.5	45.5	14.7	48.4

al. (1995) for forages, but higher than the value used in the model (3.5% per hour). On the other hand, working with fresh ryegrass, Van Vuuren *et al.* (1992; 1993) reported lower estimated values (2.4–3.5% per hour) of passage rate when lignin was used as an internal marker. Mambrini & Peyraud (1994) also reported lower values (2–2.2% per hour) of passage rate for fresh ryegrass labelled with rare earth metals. Tamminga *et al.* (1989) pointed at the high variability and low reliability of the use of the lignin fraction as an internal marker for estimating passage rate.

To investigate whether the lower prediction capacity of the model in Exp. 3 was associated with the large period of starvation (on average > 20 hours) after grazing, the root MSPE was split per evacuation. The respective root MSPE values of the observed means for OM, NDF and N were 14.8, 11.7 and 6.3% for evacuation 2 and 35.7, 43.9 and 57.2% for evacuation 3, respectively. This indicates that the observations shortly after grazing (evacuation 2) were predicted more accurately by the model than the observations after a long period of starvation (evacuation 3). Also splitting the MSPE per evacuation in Exps. 1 and 2 revealed a higher root MSPE of the observed mean after starvation (average length of starvation of 7.6 hours) than after grazing (pooled mean root MSPE for OM, NDF and N combined was 8.1 and 17.7% for evacuation 2 and 3, respectively). Important effects of starvation time on size and characteristics of rumen pool have been reported by Chilibroste *et al.* (1998a, 2000). So it is highly probable that some of the model assumptions, like constant fractional rates for production, utilization and absorption of nutrients, or fractional passage rates of solids and liquids, do not apply to our rumen conditions. It is likely that the accuracy of model prediction decreases with increasing length of the starvation period, i.e., with too large a deviation from steady state conditions. For instance, in sheep, Aitchison *et al.* (1986) observed that in the first 5 hours after a meal, between 50 and 67% of the intake of indigestible NDF was lost from the rumen, whereas only between 24 and 52% of NDF intake disappeared. A second increase in the rate of removal was observed between 15 and 24 hours after feeding. Similar observations were made for steers by Thiago *et al.* (1992). Okine & Mathison (1991) observed differences in duration and amplitude of reticular contractions during eating, ruminating and resting, possibly affecting the outflow of material from the rumen during and after feeding. The functional density of particles varies with incubation time and degradation rate (Nocek & Kohn, 1987), and the escape of particles from the rumen depends on their functional density (Kaske & Engelhardt, 1990). Consequently, when rumen particulate OM after a meal is being fermented, its specific gravity and chances of escaping from the rumen may increase or decrease, which implies that a fixed passage rate can be incorrect. During fermentation, the pH of the rumen fluid can drop due to the formation of VFA, and a pH below 6.3 can impair activities of fibrolytic bacteria, resulting in decreased rates of degradation of NDF soon after a meal when pH is lowest (Erdman, 1988). Also, absorption rates of VFA and ammonia depend on the pH of rumen fluid. For example, Dijkstra *et al.* (1993) observed that VFA absorption rates decreased as the pH increased in the range of 4.5 to 7.2. So there is ample evidence that fractional rate of passage, degradation and absorption may vary considerably as rumen conditions change.

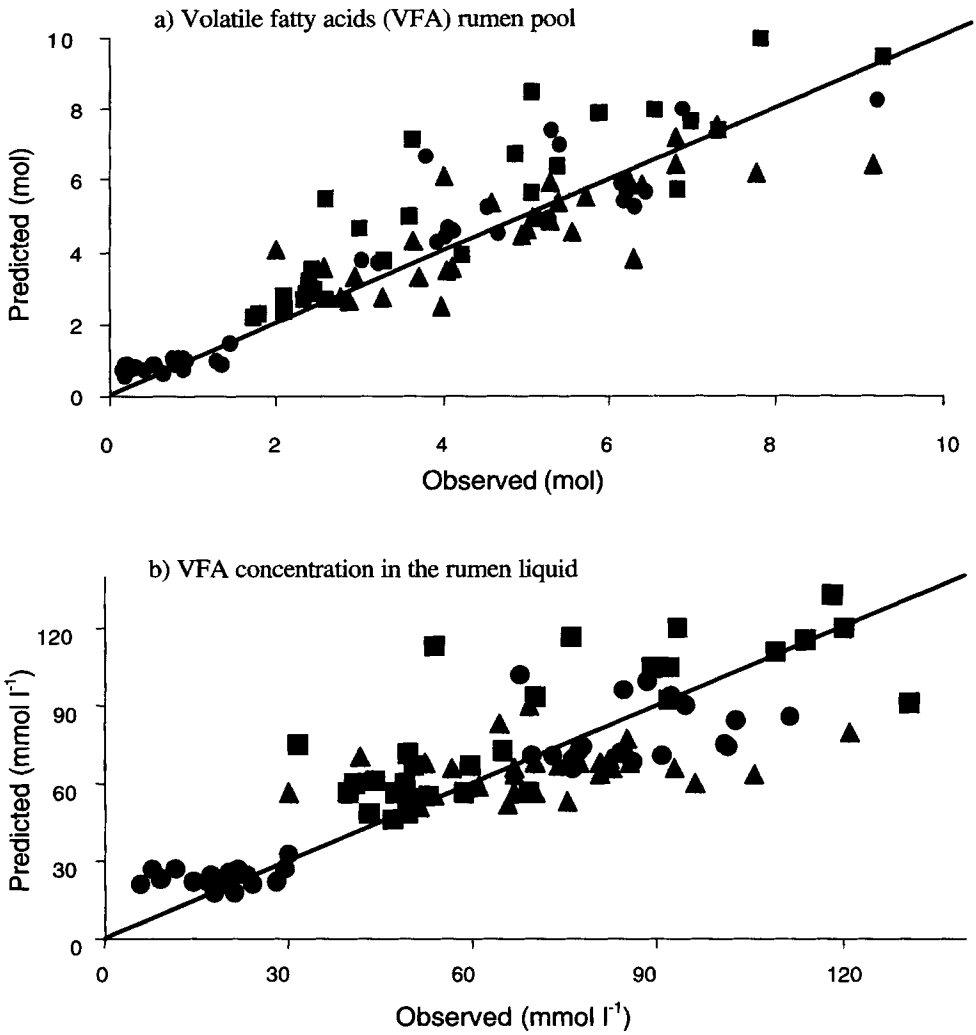

Figure 4. Predicted (line) versus observed (symbols) values for volatile fatty acids and ammonia rumen pools, and for volatile fatty acid and ammonia concentrations in the rumen liquid. ■: Experiment 1; ▲: Experiment 2; ●: Experiment 3.

Simulated and observed values of rumen fluid concentration and pool size of VFA and ammonia are plotted in Figure 4. The VFA rumen pool (Figure 4a) was predicted with a root MSPE of 33% with a large proportion of the variability explained by random variation (97%). The VFA concentration in the rumen liquid (Figure 4b) exhibited the same trend: 32 and 98% for root MSPE of observed mean and random variation, respectively. Since VFA production plays a major role in energy supply to the ruminant and hence in milk and meat production and product composition (Sutton,

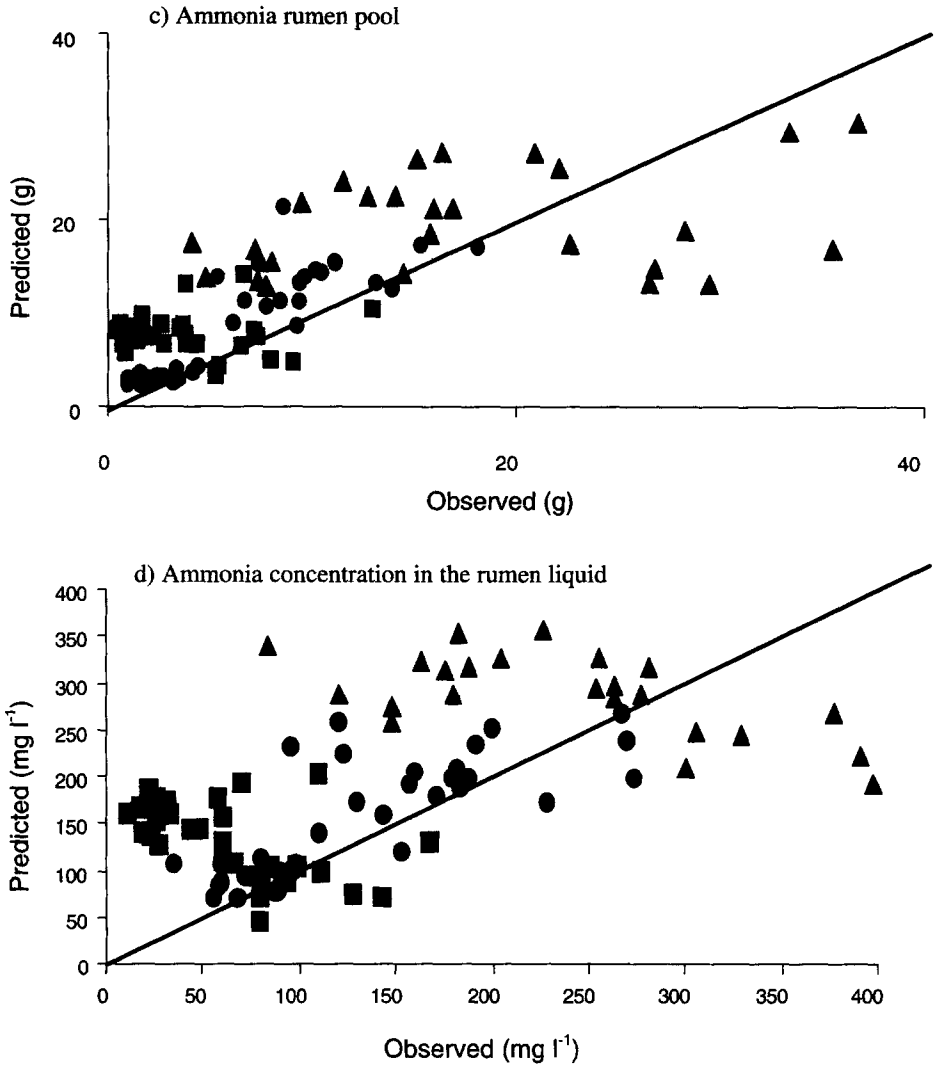

Figure 4. (continued)

1985), it has received special attention in modelling efforts (e.g. Dijkstra *et al.*, 1993). Nevertheless, the accuracy of predicting total VFA – particularly VFA molar proportions (acetic, propionic and butyric) – by existing models remains low (Bannink *et al.*, 1997; Dijkstra, 1994) and a series of suggestions has been made to improve prediction capability (Dijkstra & France, 1996). Regarding the model evaluation in the present study, it must be borne in mind that high variation coefficients were observed in the experiments (around 30%) for all the fractions measured in the rumen liquid (Chilibroste *et al.*, 1998a; 2000). So model prediction accuracy for

VFA rumen pools and VFA concentration should not be neglected. In comparison with VFA predictions, the root MSPE of the observed mean for ammonia rumen pool (79%) and ammonia concentration (69%) were larger. The large error in estimating ammonia rumen pools and ammonia concentrations suggests that the model representation of the ammonia rumen pool dynamics was not adequate (see below).

In an evaluation of a simulation model under non-steady state conditions an accurate and reliable simulation of diurnal variation of the state variables is as important as the prediction accuracy at certain fixed time points. A lack of accuracy to predict certain observed values – but with a good representation of the diurnal variation of the state variable – indicates the need for a re-parameterization or refinement of the inputs of the model. However, a good accuracy to predict the observed value at certain fixed time points but with a wrong representation of the dynamic behaviour, would indicate the need for a better representation of the whole process. The simulation of ammonia and VFA rumen pool size for the same cow in Exps. 1, 2 and 3 is shown in Figure 5.

Ammonia rumen pool exhibited a double peak in the three simulations shown in Figure 5. This pattern was consistent for all cows and in all treatments; the shape of the curve varied with initial ammonia condition, intake rate and composition of the grass ingested. In the model, three inputs for the ammonia pool were considered: (i) ammonia ingested with the diet, (ii) ammonia produced from urea transported across the rumen wall or with the saliva, and (iii) ammonia produced by fermentation of the soluble protein, which in turn arises directly from the soluble feed protein or from fermentation of the insoluble, degradable feed protein. Ammonia concentration in the grass was assumed to be 0. An analysis of the inputs during simulation revealed that the peak of the ammonia rumen pool during grazing was caused mainly by recycling of urea, and the peak after grazing mainly by fermentation of soluble protein. The simulated pattern of ammonia rumen pool and ammonia concentration (data not shown) does not agree with our observations (Chilibroste *et al.*, 2000) nor with previous research under grazing (Rearte & Santini, 1989; Van Vuuren *et al.*, 1986). In the model, the amount of urea transported was related to the concentration of ammonia in the rumen fluid and to the total N intake. There are two main factors that may contribute to incorrect predictions of urea recycling. Firstly, the maximum amount of recycled N per unit consumed N was set at 0.971 g NH_3–N per g N, which is a potentially high N recycling rate for the conditions (low N diets fed to Holstein-Zebu cattle) under which the original model was developed. This maximum value may well be too high for temperate grasses containing high levels of N fed to Holstein dairy cattle (Kennedy & Milligan, 1980). Secondly, in the original model, N recycling is related to N intake under steady state conditions, which gives rise to unbiological behaviour in the present discontinuous feeding regimens where the model predicts high urea recycling during eating and no urea recycling during periods when feed intake is zero. Recycling of urea results from urea in saliva and urea transferred by diffusion from the blood to the rumen. Saliva production is stimulated by eating and even more by rumination. Transfer of urea from the blood to the rumen is inhibited by high ruminal ammonia concentrations (Egan *et al.*, 1984). The simulation of the recycling of ammonia clearly is inadequate to represent a non-steady state.

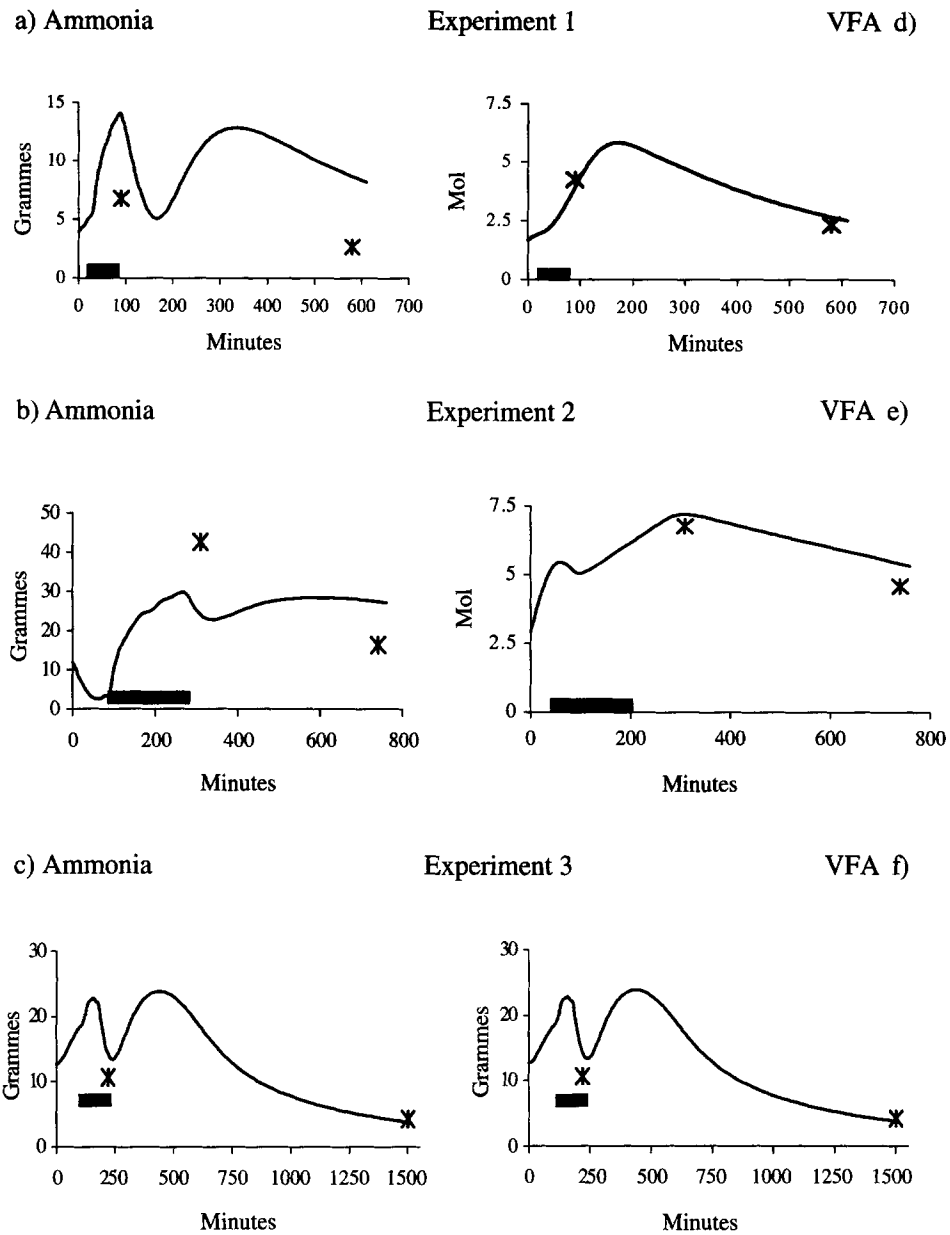

Figure 5. Simulated (line) and observed (*) ammonia and volatile fatty acids (VFA) rumen pools for Experiments 1, 2 and 3. Grazing periods represented by horizontal bars (minutes 30–90, 100–270 and 110–180 for Experiments 1, 2 and 3, respectively). Observed values are after grazing (evacuation 2; see Figure 1) and after starvation (evacuation 3; see Figure 1).

Contrary to ammonia, VFA exhibited a regular trend, which agrees with previous research findings (Chilibroste *et al.*, 2000), i.e., a slow increase of VFA rumen pool at the beginning of grazing till the degradation of degradable NDF starts, and a maximum rumen pool after the grazing is stopped. However, the model assumes that the soluble components of the ingested grass are immediately available to the micro-organisms for fermentation, which may not necessarily be the case (Chilibroste *et al.*, 1998a). Nevertheless, the trend followed by VFA rumen pool is well represented and a delay in substrate availability could be easily addressed in the model, including a particle-dynamics subroutine.

Conclusions

The non-steady state simulation model for rumen fermentation predicted the solid rumen pools (OM, NDF and N) with a favourable root MSPE of the observed mean, but still somewhat higher than desirable (> 10%). The higher root MSPE of the observed mean was largely due to the long starvation periods that followed grazing (evacuation 3, Figure 1). This increase in prediction error with increased length of starvation period indicates that better definitions of the model inputs are required, particularly fractional passage and degradation rates. Prediction of the soluble fractions of the OM rumen pool requires further evaluation. The volatile fractions (VFA and ammonia) showed considerably less satisfactory predictions than the solid fractions. VFA rumen pool was predicted with a root MSPE close to the random variation observed in the experiments. Ammonia rumen pool was poorly predicted and the ammonia representation needs considerable modification to give an accurate prediction of ammonia production and absorption under non-steady state conditions. In its present form, the model can be used to predict ruminal digestion and absorption of nutrients (except ammonia) of grazing lactating dairy cows under non-steady state conditions. But it should not be used under conditions that involve long periods of starvation.

References

Aitchison, E.M., M. Gill, M. Dhanoa & D.F. Osbourn, 1986. The effect of digestibility and forage species on the removal of digesta from the rumen and the voluntary intake of hay by sheep. *British Journal of Nutrition* 56: 463–476.

Baldwin, R.L., J.H.M. Thornley & D.E. Beever, 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. *Journal of Dairy Research* 54: 107–131.

Bannink, A. & H. De Visser, 1997. Comparison of mechanistic rumen models on mathematical formulation of extramicrobial and microbial processes. *Journal of Dairy Science* 80: 1296–1314.

Bannink, A., H. De Visser & A.M. Van Vuuren, 1997. Comparison and evaluation of mechanistic rumen models. *British Journal of Nutrition* 78: 563–581.

Bibby, J. & H. Toutenburg, 1977. Prediction and Improved Estimation in Linear Models. John Wiley & Sons, London, 188 pp.

Chilibroste, P., S. Tamminga & H. Boer, 1997. Effect of length of grazing session, rumen fill and starvation time before grazing on dry matter intake, ingestive behaviour and dry matter rumen pool sizes

of grazing lactating dairy cows. *Grass and Forage Science* 52: 249–257.

Chilibroste, P., S. Tamminga, J. Van Bruchem & P.L. Van Der Togt, 1998a. Effect of allowed grazing time, inert rumen bulk and length of starvation before grazing, on the weight, composition and fermentative end-products of the rumen contents of lactating dairy cows. *Grass and Forage Science* 53: 146–156.

Chilibroste, P., S. Tamminga & B.A. Williams, 1998b. Effect of days of regrowth of ryegrass (*Lolium perenne*) on fermentation characteristics. Comparison of the nylon bag and gas production techniques. In: E.R. Deaville, E. Owen, A.T. Adesogan, C. Rymer, J.A. Huntington & L.J. Lawrence (Eds.), *In vitro* Techniques for Measuring Nutrient Supply to Ruminants. British Society of Animal Science, Occassional Publication No 22, Edinburgh, pp. 40–43.

Chilibroste, P., B.A. Williams, S. Tamminga & S. Calabro, 1999. The use of cumulative gas production technique to characterize changes in the fermentation characteristics of rumen contents following variable periods of starvation and grazing in dairy cows. *Animal Science* 69: 647–655.

Chilibroste, P., S. Tamminga, H. Boer, M.J. Gibb & G. Den Dikken, 2000. Duration of regrowth of ryegrass (*Lolium perenne*): effects on grazing behavior, intake, rumen fill, and fermentation of lactating dairy cows. *Journal of Dairy Science* 83: 984–995.

Danfær, A. 1990. A dynamic model of nutrient digestion and metabolism in lactating dairy cows. PhD thesis National Institute of Animal Science, Foulum, 510 pp.

Dijkstra, J., 1994. Production and absorption of volatile fatty acids in the rumen. *Livestock Production Science* 39: 61–69.

Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining & S. Tamminga, 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *British Journal of Nutrition* 69: 385–396.

Dijkstra, J. & J. France, 1996. A comparative evaluation of models of whole rumen function. *Annales de Zootechnie* 45:175–192.

Dijkstra, J., J. France, H.D.S.C. Neal, A.G. Assis, L.J.M. Aroeira, & O. F. Campos, 1996. Simulation of digestion in cattle fed sugarcane: model development. *Journal of Agricultural Science* 127: 231–246.

Dijkstra, J., H.D.S.C. Neal, D.E. Beever & J. France, 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *Journal of Nutrition* 122: 2239–2256.

Egan, A.R.K. Boda & J. Varady, 1984. Regulation of nitrogen metabolism and recycling. In: L.P. Milligan, W.L. Grovum & A. Dobson (Eds.), Control of Digestion and Metabolism in Ruminants. Prentice Hall, Englewood Cliffs, pp. 386–402.

Erdman, R.A., 1988. Dietary buffering requirements of the lactating dairy cow: a review. *Journal of Dairy Science* 71: 3246–3266.

Forbes, J.M., 1995. Voluntary Food Intake and Diet Selection in Farm Animals. CAB International, Wallingford, 532 pp.

France, J., J.H.M Thornley & D.E. Beever, 1982. A mathematical model of the rumen. *Journal of Agricultural Science* 99: 343–353.

Grovum, W.L., 1995. Mechanism explaining the effects of short chain fatty acids on feed intake in ruminants – osmotic pressure, insulin and glucagon. In: W.Y. Engelhardt, S. Leonhard-Marek, G. Breves & D. Giesecke (Eds.), Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Proceedings of the Eighth International Symposium on Ruminal Physiology. Ferdinand Enke Verlag, Stuttgart, pp. 173–197.

Hungate, R.E., 1966. The Rumen and its Microbes. Academic Press, New York, 55 pp.

Kaske, M. & W. Von Engelhardt, 1990. The effect of size and density on mean retention time of particles in the gastrointestinal tract of sheep. *British Journal of Nutrition* 63: 457–465.

Kennedy, P.M. & L.P. Milligan, 1980. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. *Canadian Journal of Animal Science* 60: 205–221.

Mambrini, M. & J.L. Peyraud, 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet. *Reproduction Nutrition and Development* 34: 9–23.

Meijs, J.A.C., 1981. Herbage intake by grazing dairy cows. PhD thesis Wageningen Agricultural University, Wageningen, 264 pp.

Neal, H.D.St.C., J. Dijkstra & M. Gill, 1992. Simulation of nutrient digestion, absorption and outflow in

the rumen. Model evaluation. *Journal of Nutrition* 122: 2257–2272.
Nocek, J.E., & R.A. Kohn, 1987. Initial particle form and size on change in functional specific gravity of alfalfa and timothy hay. *Journal of Dairy Science* 70: 1850–1863.
Okine, E.K. & G.W. Mathison, 1991. Reticular contraction attributes and passage of digesta from the ruminoreticulum in cattle fed roughage diets. *Journal of Animal Science* 69: 2177–2186.
Owens, F.N. & A.L. Goetsch, 1986. Digesta passage and microbial protein synthesis. In: L.P. Milligan, W.L. Grovum & A. Dobson (Eds.), Control of Digestion and Metabolism in Ruminants, Prentice-Hall, Englewood-Cliffs, pp.196–223.
Rearte, D.H. & F.J. Santini, 1989. Digestión ruminal y producción en animales en pastoreo. *Revista Argentina de Producción Animal* 9: 93–105.
Sauvant D, J. Dijkstra & D. Mertens, 1995. Optimisation of ruminal digestion: a modelling approach. In: M. Journet, E. Grenet, M.H. Farce, M. Theriez & C. Demarquilly (Eds.), Recent Developments in the Nutrition of Herbivores, INRA, Paris, pp.143–165.
Sutton, J.D., 1985. Digestion and absorption of energy substrates in the lactating cow. *Journal of Dairy Science* 68: 3376–3393.
Tamminga, S. & A.M. Van Vuuren, 1996. Physiological limits of fibrous feed intake and conversion in dairy cows. In: A.F. Groen & J. Van Bruchem (Eds.), Utilization of Local Feed Resources by Dairy Cattle. Perspective of Environmentally Balanced Production Systems, Wageningen Pers, Wageningen, pp.19–33.
Tamminga, S., P.H. Robinson, S. Meijs & H. Boer, 1989. Feed components as internal markers in digestion studies with dairy cows. *Animal Feed Science and Technology* 27: 49–57.
Thiago, L.R.L., M. Gill & J. Sissons, 1992. Studies of method of conserving grass herbage and frequency of feeding in cattle. 2. Eating behaviour, rumen motility and rumen fill. *British Journal of Nutrition* 67: 319–336.
Van Straalen, W.M., 1995. Modelling of nitrogen flow and excretion in dairy cows. PhD thesis Wageningen Agricultural University, Wageningen, 205 pp.
Van Vuuren, A.M., C.J. Van Der Koelen & J. Vroons De Bruin, 1986. Influence of level and composition of concentrate supplements on rumen fermentation patterns of grazing dairy cows. *Netherlands Journal of Agricultural Science* 34: 457–467.
Van Vuuren, A.M., F. Krol Kramer, R.A. Van Der Lee & H. Corbijn, 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. *Journal of Dairy Science* 75: 2215–2225.
Van Vuuren, A.M., C.J. Van Der Koelen & J. Vroons De Bruin, 1993. Ryegrass versus corn starch or beet pulp fibre diet effects on digestion and intestinal amino acids in dairy cows. *Journal of Dairy Science* 76: 2692–2700.
Waghorn, G.C., 1986. Changes in rumen digesta of cows during a restricted feeding period when offered fresh red clover, lucerne, or lucerne hay. *New Zealand Journal of Agricultural Research* 29: 233–241.