MODELLING OF NITROGEN FLOW AND EXCRETION IN DAIRY COWS

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
Dr. C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 8 december 1995
des namiddags te 13.30 uur in de aula
van de Landbouwuniversiteit te Wageningen

ISN:9 17619

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Straalen, W.M. van

Modelling of nitrogen flow and excretion in dairy cows/
W.M. van Straalen. - [S.l. : s.n.]
Thesis Landbouw Universiteit Wageningen. - With ref. - With summary in Dutch.
ISBN 90-5485-475-8
Subject headings: nitrogen flow ; dairy cows

Van Straalen, W.M., 1995. Modelling of nitrogen flow and excretion in dairy cows.
In the Netherlands, the dairy husbandry is held responsible for a major part of the N-pollution of the environment. The objective of this thesis was to develop a model that predicts N flow in the animal and N excretion in faeces, urine and milk in order to reduce unnecessary N losses. The model was based on the concepts of modern protein evaluation systems, using data from nylon bag incubations in the rumen and intestine. Protein evaluation systems from different countries were compared and validated using experimental data. Different methods to estimate feed protein degradation in the rumen and digestion in the small intestine were compared and data of individual feedstuffs obtained with the nylon bag methods were collected. Experiments were carried out to obtain additional information on the organic matter fermentation and digestion of protein in the small intestine of forages, and on the change in amino acid profile of feedstuffs during rumen fermentation and small intestinal digestion. The model described the N flow in four compartments: rumen, small intestine, large intestine and metabolism. Flows were validated with experimental data. Total duodenal flow could be accurately predicted, but to predict ileal N flow a negative digestibility coefficient for endogenous protein was necessary. The model slightly overestimated faecal and urinary N excretion; milk protein production could be accurately predicted. It was concluded that N losses in dairy cows can be reduced by balancing the ratio between energy and protein in the rumen and in the metabolism, and that more information on endogenous N excretion and digestion in the gastrointestinal tract is needed.
PhD Thesis, Department of Animal Nutrition, Wageningen Agricultural University, Marijkeweg 40, 6709 PG, Wageningen, The Netherlands

STELLINGEN

1. Nieuwe voederwaarderingssystemen zijn meer gebaat bij een juiste samenhang van de onderdelen dan bij een gedetailleerde beschrijving daarvan (dit proefschrift).

2. Omdat biochemische processen verlopen via de wet van de toe- en afnemende meeropbrengst, is het niet juist om normen voor de behoefte aan voedingsstoffen uit te drukken in vaste waarden per eenheid produkt (dit proefschrift).

3. De aanname dat het aminozuurpatroon van darmverteerbaar bestendig eiwit gelijk is aan dat van het voedermiddel is een misvatting (dit proefschrift).

4. Het streven naar een hoge melkproduktie per dier is niet in strijd met het streven naar een optimale benutting van de opgenomen DVE (dit proefschrift).

5. De gebrekkige kennis van de endogene eiwitverliezen in het maagdarmkanaal bij de melkkoe bemoeilijkt een goede voorspelling van de stikstof stromen (dit proefschrift).

6. Om te komen tot een optimale benutting van stikstof door de melkkoe is voeding op basis van individuele aminozuren vereist.

7. De opvatting dat de fractionele passage- en afbraaksnelheid in de pens onafhankelijk van elkaar zijn, is wat celwanden betreft een misvatting.

8. De doelstelling van voederstrategieën zal verschuiven van maximale produktie naar maximale efficiëntie van de mineralenbenutting.

9. Een vlakkere lactatiecurve kan veel vruchtbaarheids- en gezondheidsproblemen in het begin van de lactatie voorkomen.

10. De discussie over de standaardisering van de nylon zakjes methode duurt voort totdat een goed alternatief voor deze methode voorhanden is.

11. Het opvoeden van kinderen kan men nooit goed doen, alleen zo goed mogelijk.

12. Een juist gebruik van make-up beperkt zich tot onderstreping van de natuurlijk aanwezige schoonheid.

W.M. van Straalen
Modelling of nitrogen flow and excretion in dairy cows
Wageningen, 8 december 1995.

Aan Ingrid en Nick

VOORWOORD

Het in dit proefschrift beschreven onderzoek is uitgevoerd bij het Instituut voor Veehouderij en Diergezondheid (ID-DLO) in Lelystad, waar ik als assistent-in-opleiding van de Vakgroep Veevoeding van de Landbouwuniversiteit in Wageningen gedetacheerd was. Het doel van het onderzoek was om te komen tot een model dat N-stromen in melkvee beschrijft, waardoor onnodige N verliezen zouden kunnen worden teruggedrongen. Dit model zou de basis vormen voor een nieuw eiwitwaarderingssysteem voor herkauwers in de praktijk. In een vroegtijdig stadium werd echter besloten om de ontwikkeling van een nieuw systeem ter hand te nemen. Op basis van dit nieuwe systeem (het DVE-systeem) is daarna het model voor beschrijving van de N-stromen in melkvee verder ontwikkeld. Hiervoor is een uitgebreide set van al bestaande gegevens gebruikt, die aangevuld werd met eigen onderzoeksresultaten. Vanaf deze plaats wil ik een woord van dank richten aan iedereen die een bijdrage geleverd heeft aan de totstandkoming van dit proefschrift.

Het onderzoek werd begeleid door prof. dr. ir. Seerp Tamminga als promotor en dr. Ad van Vuuren als co-promotor. Seerp, jouw stimulerende inbreng, deskundige analyse van de proefresultaten en kritische beoordeling van de manuscripten waardeer ik zeer. De avondlange discussies over de uitkomsten van de modelberekeningen brachten me steeds weer een stap verder. Ad, ik wil je vooral bedanken voor de prettige manier waarop de dagelijkse begeleiding plaatsvond, jouw kritische inbreng bij de opzet van de proeven en interpretatie van resultaten en minitieuze beoordeling van de manuscripten. Daarnaast heb ik veel voordeel gehad van het feit dat je erg handig was met de programmatuur op de computer.

Een belangrijk deel van het onderzoek bestond uit het doen van experimenten. Bij de voorbereiding en uitvoering daarvan kon ik bouwen op de instructies en hulp van vele medewerkers van het laboratorium en stofwisselingseenheid. Mijn dank gaat hierbij vooral uit naar Cees van der Koelen, die mij de details van de nylon zakjes experimenten bijgebracht heeft. De verzorging van de proefdieren was in handen van de medewerkers van de stofwisselingseenheid onder leiding van Roel Terluin. Daarnaast heb ik bij de uitvoering van de proeven hulp gehad van stagiaires, die zich altijd welwillend in de wispelturige schema's van incubaties en mest verzamelen hebben ingepast. Carolien Spira, Guido Huisman, Fokke Dooper, Wim Mostert en Jarig Jan Odinga, hiervoor mijn dank. De analyses van de monsters werden uitgevoerd op het laboratorium onder leiding van Leon de Jonge. Iedereen die hierbij geholpen heeft wil ik van harte bedanken. Zowel bij het opzetten van de proeven als de statistische analyse achteraf heb ik hulp gekregen van Paul Goedhart en Jan Kogut van de Groep Landbouw Wiskunde (GLW-DLO).

Voor de validatie van de eiwitwaarderingssystemen en de ontwikkeling en validatie van het model heb ik gebruik kunnen maken van vele gegevens van eerder uitgevoerde proeven van het ID-DLO, de Vakgroep Veevoeding, het CLO-Instituut voor de Veevoeding 'De Schothorst' en het Proefstation voor de Rundveehouderij, Schapenhouderij en Paardenhouderij (PR). Iedereen die mij hierbij geholpen heeft, wil ik

daarvoor bedanken. De hulp van Christian Salaün bij de berekeningen stel ik zeer op prijs. Verder bedank ik de vele mensen die in een directe of indirecte wijze bijgedragen tot het onderzoek en de plezierige tijd die ik bij het ID-DLO gehad heb.

During my appointment I was able to spend 3 months at the Station de Recherches sur la vache Laitière in Rennes, France. I want to thank dr. R. Vérité and his team for the instructive and pleasant time, during which I not only learned to judge the value of literature data, but also increased my knowledge of the French customs and language.

De leden van de Werkgroep Nieuw Eiwitwaarderingssysteem Herkauwers (NESH), en van de Klankbordgroep NESH wil ik bedanken voor goede samenwerking tijdens de ontwikkeling van het DVE-systeem. De snelle praktische toepassing van wetenschappelijk onderzoek heb ik gezien als een extra stimulans voor dit onderzoek.

Het laatste deel van het in dit proefschrift beschreven onderzoek werd uitgevoerd gedurende mijn aanstelling bij het CLO-Instituut voor de Veevoeding 'De Schothorst'. Hierbij wil ik het bestuur, de directie en medewerkers van De Schothorst van harte bedanken voor de mogelijkheden die zij mij geboden hebben om te komen tot een succesvolle afronding van dit proefschrift.

Het onderzoek werd gedeeltelijk gefinancierd door het Financieringsoverleg Mest- en Ammoniakonderzoek (FOMA). Het geld voor dit fonds werd beschikbaar gesteld door het Ministerie van Landbouw, Natuurbeheer en Visserij en het Produktschap voor Veevoeder (PVVr). Ik bedank de leden van de voormalige Werkgroep Mestonderzoek Melkvee (WMM) en de Werkgroep Voedingsonderzoek Herkauwers (WVH) van de Adviescommissie Veevoedkundig Onderzoek (AVO) voor de begeleiding van het onderzoek.

Het voltooien van een proefschrift is onmogelijk zonder de onvoorwaardelijke steun van het thuisfront. Lieve Ingrid, je hebt mij altijd gestimuleerd en samen met Nick de tijd gegeven die ik nodig had, zelfs tijdens de drukste perioden. Bedankt.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Dairy husbandry in The Netherlands is characterised by a high production level per cow. These production levels are obtained in an intensive production system. During the last decades the drawbacks of such an intensive production system became more apparent through an increased concern about the environmental effects. The dairy husbandry contributes for a large part to the N-pollution to the air and soil water. Emission of ammonia contributes to acidification of the soil, and loss of nitrate by leaching and surface runoff can result in higher nitrate concentration in soil water (Aarts *et al.*, 1992). To reduce the N-pollution, the Dutch government has developed targets for emissions of N into air, surface water and soil water to be met in the year 2000 (NMP Plus) and subsequently introduced matching rules (Bloem, 1992). Different means to increase N efficiency on farm level have been studied, including dietary composition, animal housing, manure storage and handling, grazing management and fertilizer application (Aarts *et al.*, 1992; Korevaar *et al.*, 1992; De Haan *et al.*, 1994).

It was estimated that in The Netherlands on average about 20% of total N intake in dairy cows was transformed into valuable products as milk and meat, and 80% was excreted in urine and faeces (Tamminga, 1992). An increase in this N efficiency by feeding can be obtained by closely matching the quantitative and qualitative supply of N in the feed with the requirements for N and thus minimizing losses during digestion and metabolisation of N. Until 1991 the DCP-system was used in the Netherlands as protein evaluation system. This system has however only limited possibilities to increase N utilisation. The main reason for this is that it describes the apparent whole tract digestion of protein and thus does not take microbial fermentation and synthesis in the rumen into account. Moreover, in practice cows were generally fed above DCP requirements. A first step to improve N efficiency in dairy cows would be by feeding cows according to this requirements, but this may under specific conditions result in a shortage of protein supply to the animal and increase the risk for disappointing milk production (Tamminga, 1986).

Further reduction in N losses without a negative effect on production requires a new protein evaluation system that describes the protein digestion and metabolism in more detail. The objective of this thesis was therefore to develop a model that predicts the N-digestion and metabolism and N-excretion with milk, urine and faeces. This model should give insight in the possibilities to reduce unnecessary N losses in dairy cows. The development of this N-flow model was simultaneously carried out with the development of a new protein evaluation system for ruminants in The Netherlands (the DVE-system; Tamminga *et al.*, 1994). Because the main aim of the DVE-system was to evaluate practical diets, it uses a rather static approach and can not be used to estimate N losses in faeces and urine directly. The N-flow model was developed to describe N digestion and metabolism in more detail and thus enabling the prediction of N-excretions.

The protein digestion and metabolism process in ruminants can be summarized as follows (Ørskov, 1982). Feed protein that is ingested by the dairy cow is subjected to microbial fermentation in the rumen, resulting in the production of small N containing compounds like peptides, amino acids and ammonia. Together with fermentation of

energy sources like carbohydrates, these products are subsequently used for the production of microbial biomass. The eventual surplus of ammonia is absorbed by the rumen wall and incorporated in urea in the liver. Unfermented feed protein and microbial protein flows to the small intestine and is partly hydrolysed to amino acids and peptides and absorbed by the intestinal wall. Endogenous protein is secreted in the small intestine and partly reabsorbed. Undigested feed, endogenous and microbial protein is further partly fermented in the large intestine resulting in production of ammonia and microbial biomass, and excreted with the faeces. Absorbed amino acids are utilized with varying efficiencies for maintenance, retention of body protein reserves or milk protein production. Absorbed N that is not recovered in milk or meat together with the eventual surplus of N in the rumen and large intestine is excreted with the urine. A small amount of N is lost in skin, hair and hoofs.

During the last decades many experiments were carried out to study the protein degradability and microbial protein synthesis in the rumen, and post rumen digestion of feed escaping rumen fermentation using *in vivo*, *in sacco* and *in vitro* techniques. These data were used as a basis for the development of new protein evaluation systems (ARC, 1984; Madsen, 1985; Vérité *et al.*, 1987). Although these systems use a comparable approach, protein values and protein requirements differed between systems (Van der Honing and Alderman, 1988). This was mainly due to differences in basal data used for the development of each system, reflecting the dietary composition in each country. Also in The Netherlands data on protein degradability in the rumen and intestinal digestibility were available (Tamminga and Ketelaar, 1988; Tamminga *et al.*, 1991). In Chapter 2 the mechanisms of protein degradation in the rumen and digestion of escape protein in the small intestine, and different methods to estimate these features were reviewed. Tabulated values for concentrate ingredients and regression equations for roughages to estimate escape protein and small intestine digestibility were established. The DVE-system was based on the principles of other modern protein evaluation systems, using the data obtained in Chapter 2 (Tamminga *et al.*, 1994). To validate various protein evaluation systems under Dutch conditions, a comparison between observed and predicted milk protein production was carried out using feeding trial carried out in The Netherlands (Chapter 3). This study also revealed that the efficiency by which absorbed protein is used for milk production was dependent on the energy to protein ratio available for milk production and the production level. This finding was further developed and incorporated into the DVE-system (Subnel *et al.*, 1994).

Under Dutch conditions fresh grass and grass silage are important sources of energy and protein for ruminants. Protein in these forages is highly fermentable in the rumen, which can result in large N losses in the rumen. The N-fertilization level, season and maturity of the sward are among the main factors influencing this degradation (Van Vuuren, 1993). A reduction in fertilization level will also result in lower N losses by leaching and enables the introduction of clover in the sward. Data on protein degradability in the rumen of clover were scarce (Beever *et al.*, 1986). The effects of season and

maturity on the degradation of protein in the rumen of intensively N-fertilized grass and moderately N-fertilized grass and clover sward were studied in Chapter 4.

For concentrate ingredients, the digestibility of escape protein in the small intestine as used in the DVE-system, was estimated by mobile nylon bag method. However, for roughages only limited data with this method were available (Tamminga *et al.*, 1994; Chapter 2). The major disadvantage of the mobile nylon bag method is that it is laborious and costly, because animals with rumen and duodenal cannula are required. Several alternatives for this method were proposed. In Chapter 5 the mobile nylon bag as method to estimate intestinal digestibility of crude protein in grass, grass silage and clover was evaluated and compared with other methods. Relationships between chemical composition of the roughages and results of the mobile nylon bag method were established.

To increase the efficiency by which absorbed protein is used for milk protein production the amino acid composition of this protein may be of importance (Rulquin and Vérité, 1993). The supply and the profile of absorbed amino acids can be altered by changing the source of escape protein in the diet. The effect of rumen fermentation and intestinal digestion on the ratio between amino acid-N and non-protein-N (NPN), and amino acid profile is not clear. In Chapter 6 an experiment is reported in which these effects were studied for some concentrate feedstuffs and roughages.

Based on data presented in the Chapters 2 to 6 and additional data from literature and unpublished experiments, the N-flow model was developed (Chapter 7). Because protein and energy digestion and metabolism interact at several levels, the fate of all components of OM in different compartments of the gastrointestinal tract (rumen, small intestine and large intestine) was described. Flows of OM and its components from these compartments and excretions of N with milk, faeces and urine were predicted and validated against experimental data.

The general discussion (Chapter 8) is focused on the further development of protein evaluation. The estimation of the protein value of feedstuffs in the current protein evaluation systems requires complex and laborious methods. These methods and alternatives are discussed. Another future development is to improve the prediction of N-excretions with milk, faeces and urine. This includes a more detailed approach of protein digestion and metabolism as described in Chapter 7. Protein values of feedstuffs estimated by the DVE-system and the N-flow model are compared. Also the possibilities to increase the N efficiency of milk protein production in dairy cows by the development of protein evaluation inwards individual amino acid evaluation is discussed. Finally, different means to reduce N losses in dairy cows by feeding management are discussed.

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CHAPTER 2

PROTEIN DEGRADATION OF RUMINANT DIETS

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Published in:
Feedstuff Evaluation (1990) (Eds. J. Wiseman and D.J.A. Cole),
Butterworths, London, pp. 57-72.

PROTEIN DEGRADATION OF RUMINANT DIETS

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Introduction

Feed protein ingested by ruminants is subject to extensive microbial degradation in the rumen. Resulting end products like ammonia and/or amino acids are subsequently incorporated to a varying extent in microbial biomass. Together with feed protein escaping degradation in the rumen, this forms the protein supply of the ruminant. Attempts have been made in recent years to bring existing information together in new protein evaluation systems for ruminants (INRA, 1978; ARC, 1980, 1984; Madsen, 1985; NRC, 1985; Vérité *et al.*,1987). The basic concept of these systems is very similar. Protein supply is estimated as the amount of protein absorbed from the small intestine, which is the sum of feed protein escaping degradation in the rumen and microbial protein formed therein and subsequently released to the lower tract, both corrected with an appropriate factor for intestinal digestion. This paper will review recently obtained information on quantitative aspects of both ruminal and post-ruminal protein digestion in ruminants.

Characterisation of feed protein

Proteins used in animal nutrition can be classified according to their solubility in water (albumins), a salt solution (globulins), alcohol (prolamines) or dilute alkali (glutelins) or on the basis of their function in the plant (enzymes, structural protein, storage protein). Nitrogen in fresh forages is 70-90% true protein and 10-30% non protein nitrogen (Tamminga, 1986). In forages three main groups of proteins occur: fraction 1 leaf protein (75% of total leaf protein), fraction 2 leaf protein (25% of leaf protein) and chloroplast membrane proteins (Mangan, 1982). Fraction 1 consists mainly of chloroplast enzymes, has a high solubility and is rapidly degraded in the rumen. Fraction 2 is a mixture of different proteins from chloroplasts and cytoplasm, with an unknown degradation rate. Chloroplast membrane proteins (mainly chlorophyll) are insoluble and degraded slowly. A small proportion of the proteins is situated in mitochondria and the nucleus for which the degradation rate is unknown. Finally some protein is linked to structural carbohydrates in the cell walls for which degradation is slow (Mangan, 1982).

Crude protein in seeds (grains, oil seeds, pulses) is mainly true protein and can be present in the husk, the pericarp or the seed itself. In husk and pericarp structural protein dominates, whereas in the seed the vast majority of protein (80-90 %) is storage protein

in the aleuron layer and in the endosperm (Ensminger and Olentine, 1978). In addition enzyme protein is found in the germ.

With regard to solubility characteristics large differences exist between different seeds (Boulter and Derbyshire, 1976). In grain seeds 10-20 % of the protein is in albumins and globulins. The remaining 80-90 % is equally distributed between prolamins and glutelins. Rice and oats are exceptions in that 70-80 % of the protein is present in glutelins and only 5-20 % in prolamins. In legume seeds 85-100 % of the protein is in albumins and globulins, none in prolamins and 0-15 % in glutelins.

Animal protein is usually present in enzymes, membranes, transport proteins (e.g. albumins in blood) or muscle (myoglobin). Depending on their origin, proteins in feeds of animal origin vary widely in their degradation properties.

Ruminal degradation of feed protein

Mechanism of protein degradation in the rumen

Anaerobic protein degradation in the rumen contains two steps, being hydrolysis of the peptide bond by proteases and peptidases (1) and decarboxylation and/or deamination of amino acids (2). The first step results in peptides and amino acids, and end products of the second step are volatile and branched chain fatty acids (VFA's and BCFA's), CO_2, and NH_3. In the rumen deamination is the most important degradative pathway of amino acid degradation (Baldwin and Allison, 1983). For a long time proteolysis was assumed to be the rate limiting step in the degradation of protein (Tamminga, 1979) but recently Chen, Russell and Sniffen (1987) provided evidence that peptide uptake was the rate limiting step and that peptides rather than amino acids are the main end products of proteolysis.

Many strains of microorganisms are involved in protein degradation and until recently it was thought that no major strain could survive on protein as the only source of energy and N (Baldwin and Allison, 1983). Nocek and Russell (1988) however, reported the isolation of a *Peptostreptococcus* with a high proteolytic activity that accounted for 10% of total colony counts and was able to grow rapidly on amino acids and peptides as the only source. Protozoa play a far less important role in the digestion of feed protein than bacteria (Baldwin and Allison, 1983; Nocek and Russell, 1988).

Measuring degradation in the rumen

Various methods exist to estimate protein (Nx6.25) degradation in the rumen, both *in vivo* and *in vitro*. A limitation of the *in vivo* method is its indirect methodology. Undegraded feed N in duodenal digesta is estimated as the difference between total N flow and microbial N flow, sometimes corrected for endogenous contaminations.

Techniques to measure duodenal digesta flow as well as microbial N have a large error, which in the calculations is transferred to undegraded feed N. *In vivo* measurements require animals equipped with duodenal cannulae, either re-entrant or T-piece. In re-entrant cannulated animals duodenal N flow can be measured and sampled with reasonable accuracy, provided the measuring period is long enough. However, this period is often restricted to 24 hours or less and the flow is subsequently corrected for an incomplete recovery of an indigestible marker. With T-piece cannulae the use of markers is the only way to estimate duodenal flow. An additional source of error may then result from samples which are not representative. If this results from a shift in the ratio between solids and liquids this can be corrected by reconstituting the samples with the double marker technique (Faichney, 1975). If the solids in the sample do not represent the solids in duodenal digesta flow this method fails.

Measuring microbial protein also requires markers. Amino acids, assumed to be present in microbes only (diaminopimelic acid, D-alanin, amino-phosphonic acid), or nucleic acids are used. Alternatives are radio-active isotopes (^{15}N, ^{32}P, ^{35}S). Estimating microbial protein without using markers is possible by comparing the amino acid profile in duodenal content with that in microbial protein and feed protein. All methods have limitations and their results often do not agree with each other (Siddons, Beever and Nolan, 1982; Theurer, 1982; Demeyer and Tamminga, 1987).

A method to estimate protein degradation in a more direct way is the nylon bag incubation technique (Mehrez and Ørskov, 1977). In this method feed samples included in nylon bags with a pore size of between 30 and 50 microns are incubated in the rumen for various lengths of time yielding a degradation curve from which the rate of degradation can be estimated (Ørskov and McDonald, 1979). Combining the rate of degradation with an appropriate rate of passage yields estimates of the 'effective' protein degradation. This method also has a number of weaknesses (Lindberg, 1985; Nocek, 1988). The first limitation is that the contents of the bags are not subjected to particle size reduction through chewing and rumination and become contaminated with microbial protein (Kennedy, Hazlewood and Milligan, 1984; Varvikko and Lindberg, 1985). This results in an overestimate of the rumen escape value which for feedstuffs low in protein may be quite significant (Nocek and Grant, 1987). A second weakness is the assumption that protein washed out of the bags is degraded instantaneously and completely. Recently it was demonstrated (Chen, Sniffen and Russell, 1987) that peptide N leaving the rumen could account for differences in effective degradation of 3-5 % units. A further complication is that the shape of the curve does not always follow the pattern of first order kinetics (Kristensen, Moller and Hvelplund, 1983; Nocek and English, 1986). Finally no agreement has been reached yet on the most appropriate rate of passage out of the rumen. Rate of passage of solids as well as liquids is influenced by level of feed intake, but the passage rate of the latter is usually much higher. Rumen clearance of solids also depends on the size of the particles and their specific weight (Hooper and Welch, 1985). The latter not only depends on the specific weight of the feed itself, but

Table 1. Regression equations to estimate rates of passage out of the rumen (from Owens and Goetsch, 1986)

Y	A	CI	RI	$(RI)^2$
Fluid	4.12	0.77	2.32	
Roughage	0.94	1.34	1.24	
Concentrates	1.30	0.61	4.88	1.25

CI= concentrate DM intake (% of body weight)
RI= roughage DM intake (% of body weight)

Table 2. Protein degradability characteristics of concentrate feedstuffs determined at IVVO

	cp	W	U	kd	B
Beans	26.5	27.5	0.8	8.24	31
Horse beans	26.3	62.8	0.3	10.82	14
Lupin	34.2	25.5	0.2	12.87	24
Peas	25.2	55.6	0.0	8.95	18
Hominy feed	18.3	27.9	1.7	7.84	32
Rice bran	14.4	32.6	6.6	9.40	31
Wheat middlings	18.4	12.8	7.2	13.44	32
Beet pulp	10.3	23.8	6.4	5.15	44
Brewers grain	24.9	4.6	30.1	5.09	65
Citruspulp	7.0	40.7	3.3	5.62	32
Corn gluten feed	21.6	44.9	5.5	5.15	32
Palmkernels	10.4	8.0	6.5	2.39	68
Soya beans (raw)	40.6	33.9	0.0	10.24	24
Soya beans (toasted)	39.0	7.4	0.0	6.76	44
Babassu meal	20.2	3.0	9.5	3.35	66
Coconut meal	21.5	13.9	3.2	3.03	58
Cottonseed meal	48.6	13.4	1.8	7.58	39
Groundnut meal	57.0	22.3	1.3	9.39	31
Linseed meal	33.4	17.2	4.1	5.04	48
Nigerseed meal	36.1	9.4	5.4	10.56	36
Palmkernel meal	15.1	8.8	6.7	3.47	60
Rapeseed meal	36.9	21.2	5.9	13.84	29
Ricebran meal	14.2	4.3	17.6	6.18	56
Soyabeanmeal	49.5	6.2	0.1	8.25	40
Sunflowerseed meal	37.2	14.7	3.3	14.68	27
Feathermeal	88.9	13.3	9.5	0.95	76
Meatmeal	60.4	36.2	11.8	1.66	53
Alfalfa meal	16.0	26.0	19.9	5.44	48

cp = crude protein fraction in dm (%).
W = washable crude protein fraction (%).
U = undigestible crude protein fraction (%).
k_d = degradation rate (%/hour).
B = effective bypass protein fraction (%) assuming k_p = 0.06 (%/hour).

also on microbial activity on their surface (Sutherland, 1986). Degree of digestion also seems to have an influence. Recently it was demonstrated in dairy cows that undigestible material (IADF) was passing out of the rumen at a much faster rate than digestible material (Tamminga *et al.*, 1989).

In the ARC approach (ARC, 1984) passage is restricted to the small particles and rates of 0.02 to 0.08 fractions per hour were suggested for different diets and levels of intake. In the Nordic and French protein evaluation system for ruminants (Madsen, 1985; Vérité *et al.*, 1987) a rate constant of 0.08 and 0.06 is suggested for all diets and all levels of intake. In the Cornell Net Carbohydrate/Protein system for evaluating cattle diets, concentrate ingredients are classified on the basis of their weight and the assumed rumen passage rate at maintenance level of feed intake varies between 1 and 3.5 % per hour. In a review Owens and Goetsch (1986) derived regression equations for the passage of fluid, roughage particles and concentrate particles. The equations proposed by them are summarised in Table 1.

A number of *in vitro* methods to estimate protein degradation in the rumen have also been developed. Initially N-solubility in rumen fluid was proposed (Wohlt, Sniffen and Hoover, 1973). The results varied however with the solvent used (Crooker et al., 1978). The method does not give information on rumen degradation characteristics of the non soluble part of the protein, which may be quite variable. A further development was incubation *in vitro* with rumen fluid using ammonia or amino acids release as an indicator for microbial degradation (Broderick, 1982) or with purified proteolytic enzymes (Pichard and Van Soest, 1977).

Although nylon bag incubations are too complicated and laborious to be used as screening method for large numbers of feedstuffs, because of its directness it is felt that this technique is at present the most reliable method, provided a standardized procedure is used. Presenting results should also be standardized. With nylon bag incubation studies, protein in ruminant feeds can be separated in a washable fraction (W), which can be washed out of the bags without rumen incubation, an undegradable fraction (U), determined with a long term (10 days) rumen incubation and a degradation rate (k_d) for the unsoluble degradable fraction (D=100-W-U), estimated from the degradation curve and if relevant a lag period. It is recommended that data be presented in this way (Table 2). The advantage is that effective degradation or bypass (B) can easily be recalculated in case rumen passage rates need adjustment. Figures in Table 2 are based on assumed rate of passage of 0.06/hr.

Effective degradation of protein in feedstuffs

Data on effective protein degradation of concentrate feedstuffs, determined by nylon bag incubations in different laboratories with at least six different feedstuffs each, were collected (W.A.G. Veen, unpublished; J. Oskamp, unpublished; Ørskov, 1982; Cronje, 1983; Shibui *et al.*, 1983; De Boever *et al.*, 1984; Madsen and Hvelplund, 1985; Barrio,

Table 3. Mean bypass protein of concentrate feedstuffs obtained from different laboratories, before and after correction for laboratory influence

Feedstuff	cp	n	Before		After	
			B	CV	B	CV
Barley	11	8	28	32	34	12
Corn	10	8	58	24	57	21
Oats	12	5	19	42	24	36
Milo	10	3	58	11	57	10
Rye	11	3	19	26	22	34
Wheat	14	7	23	26	29	25
Beans	26	4	32	42	33	6
Horse beans	29	4	18	40	19	27
Lupin	33	5	14	52	22	25
Peas	24	6	20	28	24	24
Hominy feed	14	2	48	47	38	24
Cassava meal	3	2	32	20	40	26
Rice bran	15	4	31	48	34	6
Rye middlings	16	1	42	-	32	-
Wheat middlings	19	5	24	25	31	15
Wheat bran	18	3	25	40	34	5
Beetpulp (10-15% sugar)	11	7	49	34	49	12
Beetpulp (20-25% sugar)	14	4	43	26	39	25
Brewers grains	27	7	67	19	61	14
Citrus pulp	7	3	37	84	37	29
Corn gluten meal	65	9	75	11	69	7
Corn gluten feed	22	7	24	48	32	13
Cottonseed	18	2	22	55	27	12
Linseed	23	3	28	42	27	43
Rapeseed	21	1	13	-	22	-
Palmkernels	10	1	68	-	68	-
Soyabeans (raw)	39	6	19	50	25	16
Soyabeans (toasted)	40	3	41	55	41	11
Sunflowerseed	19	2	8	10	20	11
Babassu meal	20	2	76	18	62	10
Coconut meal	22	6	60	23	57	7
Cottonseed meal	43	8	44	25	43	11
Groundnut meal	52	7	21	50	26	13
Linseed meal	36	9	40	33	42	16
Nigerseed meal	36	1	36	-	36	-
Palmkernel meal	16	5	61	35	58	11
Rapeseed meal	39	7	29	24	34	17
Rice bran meal	14	2	53	9	54	4
Sesamseed meal	46	2	28	66	33	6
Soyabean meal	50	11	36	32	39	8
Sunflowerseed meal	37	10	23	40	28	19
Soya hulls	16	2	53	26	43	1
Blood meal	89	2	79	7	75	7
Feather meal	90	3	80	6	66	16
Fish meal	73	11	57	18	56	13
Meat meal	60	6	51	14	49	15
Meat and bone meal	52	3	44	24	45	23
Alfalfa meal	17	5	44	33	45	7
Grass meal	13	2	35	47	43	6

cp = crude protein content in the dry matter (%). B = effective protein bypass fraction (%).
n = number of laboratories. CV = coefficient of variation.

Goetsch and Owens, 1986, Vérité *et al.*, 1987; Erasmus, Prinsloo and Meissner, 1988; Tamminga and Ketelaar, 1988; Susmel *et al.*, 1990). Between laboratories differences were substantial, but the sequence of degradation of the feedstuffs was usually very similar. Regression equations were calculated between the results in each data set and those obtained at IVVO with the same ingredients. In this regression analysis the products of animal origin were excluded because they usually did not fit the regression equation. R squared for the regression equations ranged between 0.63 and 0.96. From these equations corrected degradation values were calculated. This reduced the average coefficient of variation from 32 % to 16 % (Table 3).

Protein escape values for roughages, based on nylon bag incubations are limited (Filmer, 1982; Cronje, 1983; Madsen and Hvelplund, 1985; Vérité *et al.*, 1987). The composition of forages varies much more than that of concentrate feedstuffs and depends on species, maturity, fertilization level, season, soil type and weather conditions. Degradation of forage protein, using nylon bag incubations was studied extensively in our institute. A total of 28 samples of fresh grass (Van Vuuren and Tamminga, unpublished observations), 36 samples of grass silage and 10 samples of grass hay (Tamminga, Ketelaar and Van Vuuren, 1991; Bosch and De Visser, unpublished observations) were studied. Important sources of variation were N content and date of harvest for all forage samples. Silages varied also in dry matter content.

Material was chopped at 1 cm before incubation. Grass samples were incubated in grass fed cows, whereas silage samples and hay samples were incubated in cows fed diets consisting of hay and concentrates. Measurements were on washable fraction (W), undegradable fraction (U) and rate of degradation (k_d). For all forages a ruminal passage rate of 4.5 % per hour was assumed.

The results (Table 4) showed significant influences on W, U and k_d in all forages for N content, NDF content and day of harvest and for silage an additional influence for dry matter content. For fresh grass, grass silage and grass hay an average protein bypass value (B) was found of 29, 22 and 42 % at an average N content of 35, 36 and 32 g/kg dm respectively. Again significant influences of N content and day of harvest were observed for all forages with additionally an influence of dry matter content for silages. Estimating protein degradation in corn silage was unsuccessful, because of severe contamination of the bag contents with bacteria during the first 24 hours of the incubation. From the disappearance of N between 0 and 48 hours it was estimated that the proportion escaping degradation in the rumen was close to 25 %, of which almost half was undegradable (Tamminga and Ketelaar, 1988).

Manipulation of protein degradation in the rumen

As was stated before, protein in ruminant feeds can be characterised as containing an undegradable fraction (U), a (slowly) degradable insoluble fraction (D) and a (rapidly)

degradable soluble fraction (W). Degradability of feed protein is thus determined by the fraction U, degradation by the ratio of the rate of degradation (k_d) and the rate of passage out of the rumen (k_p).

Manipulation of degradability is restricted to reducing the size of U, manipulation of degradation can be achieved both by changing the size of U and by changing the ratio k_p/k_d. Means to manipulate degradability of individual feedstuffs are changing growing conditions (forage) or processing (physical, chemical). Manipulation of degradation is possible through the same methods, but additionally feeding management factors are important. For mixed diets the same manipulation principles are applicable, but in addition protein degradation of the diet can be manipulated by selection of its ingredients for a high or a low degradability.

Table 4. Relationships between degradability characteristics, chemical composition and season of harvesting of roughage feedstuffs

Roughage	Y	a	b1=dm	b2=cp	b3=day	R^2
Fresh grass	U	10.2		-0.037	0.022	0.62
	k_d	8.9		0.027	-0.034	0.44
	B	38.6		-0.080	0.070	0.73
Grass silage	W	81.5	-0.040		-0.093	0.63
	U	19.0	0.006	-0.066	-0.025	0.58
	k_d	2.5	-0.008	0.035		0.43
	B	19.8	0.031	-0.077	0.071	0.81
Grass hay	W	12.5		0.098		0.49
	U	24.2		-0.073		0.81
	k_d	5.9		0.007	-0.033	0.55
	B	50.3		-0.110	0.131	0.80

b1 = coefficient for dry matter fraction in product (g/kg).
b2 = coefficient for crude protein fraction (g/kg DM).
b3 = coefficient days elapsed since 1st of April.
W = washable crude protein fraction (%).
U = undigestible crude protein fraction (%).
k_d = degradation rate (%/hour).
B = effective protein bypass fraction (%).

Manipulation of protein degradability

Research at our institute (Van Vuuren and Tamminga, unpublished observations) illustrated that degradability of protein in forages can be influenced by manipulation the growing conditions, of which level of N fertilisation, maturity and season are important factors.

A high N fertilization of grasses, mainly consisting of perennial ryegrass, resulted in a decreased size of U, hence degradability increases. Increasing stage of maturity had the opposite effect as did progression of the season. Similar observations were made for silages and grass hays (Tamminga, Ketelaar and Van Vuuren, 1991). Sun drying, leading to wilted silage or hay did increase the size of U as well and therefore resulted in a decreased degradability. Nocek and Grant (1987) observed a similar tendency for orchard grass and timothy, but alfalfa and clover showed an opposite trend.

During the ensiling process, artificial drying of roughages and processing of seeds, protein can be damaged by heat, leading to less soluble protein and probably to an increased size of fraction U. Acid detergent insoluble N (ADIN) has been proposed as a measure of such damage (Thomas *et al.*, 1982).

Manipulation of protein degradation

N fertilization increases crude protein content but also the size of fraction W and the rate at which fraction D is degraded in the rumen. This leads to a reduced protein escape from the rumen. In grass with a relatively high protein content less protein is bound to the structural carbohydrates (Moller, 1985; Tamminga, Ketelaar and Van Vuuren, 1991). In nylon bag incubation studies Nocek and Grant (1987) observed large differences in rate of protein degradation between grasses (orchard grass, timothy) and legumes (alfalfa, clover); N in legumes disappeared at a much faster rate. Tamminga, Ketelaar and Van Vuuren (1991) found that when the season progressed the degradation of protein in fresh grass and grass silage decreased. However, Beever *et al.* (1986) in experiments with sheep found no difference in protein degradation *in vivo* between ryegrass and clover and season of harvesting.

Grass silage can be made from freshly cut grass or after wilting. During the wilting period plant proteases are active, particularly under moist conditions and a high temperature (McDonald, 1982). Changes in the nitrogen fraction as result of the ensiling process itself depend on the type of fermentation but can be extensive, resulting in a high N-solubility. In a stable silage lactic acid bacteria which have very little proteolytic and deamination activity predominate. In an unstable silage *Clostridia* may proliferate and their activity results in severe protein breakdown (McDonald, 1982). Wilting grass before ensiling, increases the osmotic pressure and therefore reduces the fermentation of protein in the silage (Tamminga, Van Vuuren and Ketelaar, 1991).

Protein breakdown in silage can also be reduced by the application of formic acid, formaldehyde or a combination of both. Addition of formic acid quickly reduces the pH and establishes a stable silage, but has no influence on the protein degradation in the rumen (Chamberlain, Thomas and Wait, 1982; McDonald, 1982). Application of formaldehyde in grass silage has a sterilizing effect on *Clostridia* and decreases protein degradation in the silage as well as in the rumen through the formation of bonds between the formaldehyde and proteins (McDonald, 1982). With fresh forage (lucerne and grass), formaldehyde treatment also resulted in a decreased rumen degradation of the protein (Beever *et al.*, 1987).

Due to proteolysis during wilting protein in grass hay has a higher soluble fraction than protein in fresh grass. This is more than compensated for by a lower degradation rate as well as a higher undegradable fraction, thus a smaller effective degradation (Tamminga and Ketelaar, 1988), because of the absence of proteolysis during storage. Grass hay has a smaller soluble fraction and degradation rate than grass silage, but a higher undegradable fraction, which also results in a higher protein escape (Janicki and Stallings, 1988; Tamminga, Ketelaar and Van Vuuren, 1991).

Protein degradation of concentrate feedstuffs can be influenced by processing, which normally takes place with cereals and oilseeds and treatment with chemical agents. Oilseed extraction reduced protein degradation in the rumen; expelling was more effective than solvent extraction (Goetsch and Owens, 1985; Stern, Santos and Satter, 1985; Broderick, 1986; Pena, Tagari and Satter, 1986). According to Satter (1986), heat treatment increases both the undegradable and undigestible protein fraction and the maximum intestinal supply of absorbable protein depends on the time and temperature of the treatment. Formaldehyde treatment of soyabean meal and rapeseed meal resulted in an increased ruminal escape (Rooke, Brookes and Armstrong, 1983; Crooker *et al.*, 1986). Alcohol treatment changes the structure of proteins, leading to a more hydrophobic character. This also causes a decreased rumen degradation (Lynch *et al.*, 1987; Van der Aar *et al.*, 1984).

Manipulation of protein degradation can also be achieved by feeding management, like changing the ratio, sequence, level and frequency of feeding concentrate and roughage (Tamminga, 1979).

Feeding roughage rich diets generally results in a faster ruminal protein degradation than diets rich in concentrates which is probably due to a higher ruminal pH that stimulates microbial activity. In concentrate based diets more protein is in small particles, which can escape rumen fermentation when fed in large amounts at a time. (Ganev, Ørskov and Smart, 1979; Owens and Bergen, 1983; Zinn and Owens, 1983). However, Tamminga (1981) found no difference in protein escape between diets differing in their roughage to concentrates ratio. This would suggest that diets which are broken down slowly stay longer in the rumen. Within roughage based diets, protein degradation was demonstrated to be higher in animals fed fresh grass than in diets based on hay (Tamminga, unpublished observations). This can be due to the proteolytic activity of plant

proteases of fresh grass in addition to the bacterial activity or due to a shift in the microbial population in favour of proteolytic bacteria.

The effect of both frequency of feeding and feed intake level on protein degradation seems restricted to concentrate based diets and becomes evident mainly at a high level of feed intake. More frequent feeding stabilizes rumen fermentation and increasing the feed intake level can increase ruminal passage rate of particles and fluid and alter microbial proteolytic activity (Robinson and Tamminga, 1984).

Feeding concentrate based diets more frequently at a high level of feed intake resulted in an increased protein degradation, while with diets consisting mainly of roughages no effect was recorded (Tamminga, 1981; Robinson and Sniffen, 1985; McAllan, Lewis and Griffith, 1987). Firkins *et al.* (1986) and Rahnema *et al.* (1987) found no effect of feeding level on protein degradation in experiments with steers. With dairy cows fed concentrate based diets, Tamminga, Van der Koelen and Van Vuuren (1979) observed a decreased protein degradation with increased feed intake, while Robinson, Sniffen and Van Soest (1985) could not detect any effect with roughage based diets.

The effects of protein content of the diet on the effective protein degradation are conflicting. Barney *et al.* (1981) and Kirkpatrick and Kennelly (1987) showed an increased degradation with increasing protein content in the diet, while Forster *et al.* (1983) and Murphy and Kennelly (1987) found no effect.

Intestinal digestion of protein

Mechanism of intestinal protein digestion

Reduction of the degradation of feed protein in the rumen will only be beneficial to the animal when protein escaping degradation in the rumen is absorbed from the small intestine (SI). Apparent absorption gives an estimate of the amount of protein which becomes available for the animal's organs and tissues, true absorption gives an indication of the true protein value of the feed. The difference is endogenous protein, which not only means a direct protein drain from the animal, but also an indirect loss as its replacement needs considerably more protein than is excreted as such. Recently it was stated (NRC, 1985) that faecal excretion of endogenous protein was closely related to the amount of dry matter excreted in the faeces rather than the dry matter ingested and that replacement of the excreted protein would require 1.5 times that amount to be absorbed.

Although the large intestine is believed not to absorb amino acids, protein escaping small intestine digestion can be made valuable for the animal through microbial fermentation to NH_3, which can be taken up by the large intestine, and subsequently used by rumen microorganisms.

Measuring intestinal digestion

Various ways are possible to estimate intestinal digestion, of which the majority are only applicable to the mixture of undegraded feed protein, microbial protein and endogenous protein. Duodenally cannulated animals, preferably with cannulae in the beginning and at the end of the SI, provide information on the apparent absorption of intestinal protein. Alternatives are to infuse protein sources in the abomasum or beginning of the SI and to measure the increased faecal protein output (Schwarting and Kaufmann, 1978) or increased ileal protein flow (Hvelplund, 1985). The advantage of such methods is that information on the absorption of protein in individual feedstuffs can be obtained. A limitation is that infusion is not restricted to protein, but that the feedstuffs also contain dry matter other than protein, which causes the release of extra endogenous protein. This is likely to result in an underestimation of the true absorption, particularly if the increased ileal flow is measured. An alternative method is by regression (Van Bruchem *et al.*, 1985).

A recently developed method is the mobile nylon bag technique (Sauer, Jorgensen and Berzins, 1983). With this method small quantities of feedstuffs are included in small nylon bags, incubated in the rumen and, subsequently, introduced into the beginning of the small intestine and after passage through the intestinal tract, recovered from the faeces or ileum. This technique has a high potential, because the capacity to evaluate individual feedstuffs is quite high. In research in dairy cows at IVVO, four bags could be introduced in the beginning of the small intestine every 20 minutes over a period of more than 24 hours, resulting in a daily capacity of up to to 300 bags.

Intestinal protein digestion of feedstuffs

The apparent absorption of total amino acid nitrogen (AAN) from the small intestine ranges from 65-75% and is higher than the non ammonia nitrogen (NAN) absorption (55-70%). It is generally thought that essential amino acids (EAA) are absorbed to a greater extent than non essential amino acids (NEAA) but literature data are conflicting (Santos, Stern and Satter, 1983; Hvelplund, 1984; Moller, 1985; Stern, Santos and Satter, 1985). From the regression technique (Van Bruchem *et al.*, 1985) it appears that true digestion of concentrate ingredients is usually high. This observation was confirmed by the results of infusion studies (Schwarting and Kaufmann, 1978; Hvelplund, 1985) as well as mobile nylon bag studies (Robinson and Tamminga, 1984; Hvelplund, 1985; De Boer, Murphy and Kennelly, 1987).

Results of intestinal digestion studies on feedstuffs with the mobile nylon bag technique were collected from different laboratories (Hvelplund 1985; De Boer, Murphy and Kennelly, 1987; Vérité *et al.*, 1987; Tamminga and Ketelaar, 1988) and are presented in Table 5. In general, roughages show a much lower intestinal protein digestion than concentrate ingredients. This difference probably result from the fact that leaf protein in

Table 5. Mean protein digestibility in the small intestine determined by the mobile nylon bag and infusion technique of feedstuffs from different laboratories

Feedstuff	Mobile nylon bag Mean	CV	n	Infusion Mean	CV	n
Barley	90	-	1			
Corn	97	-	1			
Wheat	94	-	1			
Horse beans	91	4.0	2			
Lupin	98	-	1			
Wheat middlings	79	8.5	2			
Dried beet pulp	89	-	1			
Brewers grains	93	1.6	2			
Corn gluten feed	89	4.0	2			
Corn gluten meal	99	1.8	2			
Palm kernel	56	-	1			
Rapeseed	50	-	1			
Soyabean (raw)	88	11.7	2			
Soyabean (toasted)	84	-	1			
Coconut meal	92	3.1	3	85	-	1
Corn germ meal	91	-	1			
Cottonseed meal	87	-	1	82	19.8	2
Groundnut meal	97	0.3	2	90	-	1
Linseed meal	88	2.6	2	83	5.1	2
Palmkernel meal	82	7.6	2	54	-	1
Rapeseed meal	79	7.3	4	78	17.0	3
Soyabeanmeal 45% cp	99	0.3	2			
Soyabeanmeal 55% cp	98	2.1	3	84	3.6	3
Soyabean/rapeseed meal	97	-	1			
Sunflowerseed meal	91	4.7	3	71	8.0	2
Soya hulls	68	-	1			
Fish meal	92	2.2	3	87	2.7	3
Meat meal	72	-	1			
Meat and bone meal	65	-	1			
Fresh grass	63	7.2	6			
Alfalfa	73	6.4	3			
Alfalfa hay	64	34.3	2			
Alfalfa silage	85	-	1			
Corn silage	74	-	1			

CV = coefficient of variation. = number of laboratories.

roughages is already largely degraded in the rumen and the protein escaping is associated with cell walls which cannot be digested in the small intestine and only to a small extend in the large intestine. In concentrate ingredients protein escaping degradation in the rumen is mainly storage protein, not protected by cell walls.

Data obtained with the infusion technique (Schwarting and Kaufmann, 1978; Kaufmann 1979; Hvelplund 1985) were consistently lower than the digestibilities obtained by the mobile nylon bag technique, probably due to increased endogenous secretions with the infusion technique. The mobile nylon bag technique can give overestimation when bags are recovered from the faeces because of disappearance of N in the large intestine (Hvelplund, 1985; Voigt et al., 1985). *In vivo* disappearance of NAN and AAN from the large intestine ranges between 11 and 34 and from -3 to 37% respectively (Santos, Stern and Satter, 1983; Moller, 1985). However for the majority of feedstuffs the fraction of protein that has not disappeared in the small intestine is rather small and the overestimation limited.

Manipulation of intestinal digestibility

The disappearance of protein from the intestine can be decribed by digestibility (1 minus the undigestible fraction) and digestion (the fraction which has actually disappeared). Because of the high potential of the intestine to digest and absorb nitrogen, digestion approaches digestibility and therefore the distiction seems rather theoretical. At a high level of feed intake difference becomes apparent but remain small. At a high level of feed intake Hvelplund (1984) found a decreased apparent N digestion in the small intestine and Tamminga and Ketelaar (1988) reported a reduced intestinal digestion measured with the mobile nylon bag technique, due to a much shorter transit time.

Frequency of feeding and level of feed intake can alter the protein escape from the rumen. The effect of rumen retention time on intestinal digestion of escaped feed protein is variable and depends on the feedstuff (Hvelplund, 1985; Rooke, 1985; Voigt *et al.*, 1985; De Boer, Murphy and Kennelly, 1987). Results from mobile nylon bag experiments from IVVO indicate that incubation in the rumen for 6 or 18 hours had no significant effect on intestinal digestion. Treatment of feedstuffs with heat or chemical agents can reduce protein degradation in the rumen and also intestinal digestion when feedstuffs are overprotected. Hvelplund (1984, 1985) showed decreased digestion of formaldehyde treated soyabean meal and heat damaged fishmeal and Rooke, Brookes and Armstrong (1983) found the same effect with formaldehyde treated soyabean meal and rapeseed meal. Heat treatment of whole soyabeans on the other hand resulted in an increased small intestine digestion of total N and AAN, which could be due to denaturation of the trypsine inhibitor (Stern, Santos and Satter, 1985). Treatment of roughages with formic acid or formaldehyde had no effect on the apparent total tract digestion of the protein (Chamberlain, Thomas and Wait, 1982).

Conclusion

Measuring of feed protein escape from the rumen and subsequent digestion in the intestine should be standardized. When using nylon bag incubations, protein should preferably be descibed by a washable fraction (W), undegradable fraction (U) and a degradation rate (k_d) of the unsoluble degradable fraction (D=100-W-U). The degradability of protein in feedstuffs is detemined by the fraction U; the actual degradation depends on the degradability and the conditions under which degradation takes place, which determine the ratio degradation rate/passage rate (k_d/k_p). When measured under standardized conditions protein degradation in both concentrate ingredients and roughages is variable. For measurement of the intestinal digestion of escape protein the mobile nylon bag technique is at present the most powerfull method. Based on limited data, bypass protein from concentrate ingredients has a high digestibility; that of roughages is lower and more variable.

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CHAPTER 3

VALIDATION OF PROTEIN EVALUATION SYSTEMS BY MEANS OF MILK PRODUCTION EXPERIMENTS WITH DAIRY COWS

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Published in:
Netherlands Journal of Agricultural Science (1994) 42, 89-104.

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Abstract

Protein evaluation systems (CP, DCP, PDI, AAT, AP, MP, AAS and DVE) were validated using data from 15 production experiments with dairy cows, carried out in The Netherlands. Only treatments that were deficient in protein according to at least one system were selected. Average production was 31.2 kg fat and protein corrected milk d^{-1} and 989 g milk protein d^{-1}. The observed milk protein production was compared with milk protein production, predicted from the protein supply and requirements in each system.

The difference between observed and predicted milk protein production expressed as the absolute and relative prediction error was smallest for the DVE-system (-2 g d^{-1}; respectively 5.7%) and increased in order of CP (-22 g d^{-1}; 6.7%), PDI (-19 g d^{-1}; 7.8%), DCP (-44 g d^{-1}; 8.8%), AP (-37 g d^{-1}; 9.3%), AAS (100 g d^{-1}; 11.7%), AAT (112 g d^{-1}; 13.4%) and MP-system (204 g d^{-1}; 22.9%). Predictions can be improved when a variable efficiency of milk protein production is used. In the DVE-system the observed efficiency decreased with increasing protein to energy ratio in the diet and milk production level. It was concluded that under Dutch conditions the prediction of milk protein production decreased in order of the DVE, CP, PDI, DCP, AP, AAS, AAT and MP-system.

Keywords: dairy cows, protein, evaluation systems

Introduction

Protein evaluation for ruminants had until recently in many countries been based on digestible crude protein (DCP). Although the system was satisfactorily used, shortcomings became more and more apparent. A major disadvantage of the DCP-system is that it does not take into account protein degradation and microbial protein synthesis in the rumen. With the DCP-system it is therefore not possible to use recently acquired knowledge of the protein digestion process to improve efficiency of N utilisation in dairy cows. During the last decades new protein evaluation systems for ruminants, describing the protein digestion process more detailed than the DCP-system, have been developed and introduced (Van der Honing and Alderman, 1988).

The protein unit (PU) of a feedstuff and the requirements of PU in these systems are expressed as true protein absorbable from the small intestine. Examples are: PDI (Vérité *et al.*, 1987), AAT (Madsen, 1985), AP (NRC, 1985), MP (AFRC, 1992), AAS

(Ausschüss für Bedarfsnormen, 1986) and DVE (Tamminga *et al.*, 1994) (for explanation of abbreviations of systems see Table 2). All these systems use a factorial approach in which PU originates from feed protein escaping rumen degradation (escape protein) and from microbial protein synthesized in the rumen. The PU requirement is separated into requirements for milk production, maintenance, gestation and, in case of the MP and DVE-system, retention or mobilisation of body reserves. Because systems use different factors and tables to calculate PU supply and requirements, the optimal crude protein (CP) concentration and escape protein fraction in the diet vary considerably between systems (Waldo and Glenn, 1984; Alderman, 1987).

Although numerous experiments were carried out to quantify the effect of protein feeding or post-ruminal infusion on milk protein production, only a limited number of these data were used to directly validate protein evaluation systems (Jarrige and Alderman, 1987). Generally PU intake in the newly developed systems showed a better relationship with milk protein production than CP or DCP intake (Thuen and Vik-Mo, 1985; Vik-Mo, 1985; Bricenco *et al.*, 1988). The difference between predicted and observed milk protein production varied between systems and experiments (Vik-Mo, 1985; MacRae *et al.*, 1988; Sloan *et al.*, 1988; Garnsworthy, 1989; Cody *et al.*, 1990; Broderick *et al.*, 1990; Robinson *et al.*, 1991; Susmel *et al.*, 1991). This variation could be due to differences in experimental design, type of feedstuff and production level and type of animals used.

The objective of the present study was to validate protein evaluation systems by comparing the predicted to the observed milk protein production, measured in production experiments carried out in The Netherlands with high yielding dairy cows having a PU intake below PU requirements in at least one system.

Material and methods

Production experiments

Data from 15 production experiments with early to mid-lactating dairy cows were available. The experiments were carried out at four different locations. Information on the number of animals, treatments, experimental weeks and type of diets is given in Table 1. In the experiments Dutch Friesian and Holstein Friesian cows were used which were housed in a loose housing or tie stall and fed individually. Roughage consisted of fresh grass, grass silage, maize silage or a mixture of these roughages (Table 1). In each experiment roughage intake, concentrate intake, milk production, milk protein and fat content and body weight of each cow were measured weekly.

Table 1. Number of animals, treatments and duration of the experiments

Experiment nb.	ref[a]	Number of animals	Roughage type[b]	Treatments: Description[c]	Total	Number Selected[d]	Roughage: Concentrate	Weeks[e] PP	MP
1	1	59	GS	%ECP	4	0	45:55	3	21
2	2	28	GS	CP	2	0	50:50	3	17
3	2	52	GS,MS	%ECP,GS:MS-ratio	8	0	40:60; 44:56	3	15
4	3	28	G,MS	G:MS-ratio,feeding method	4	1	80:20	2	6
5	3	24	G,MS	G substitution	4	3	80:20; 60:40	2	6
6	4	92	GS	%ECP	4	2	45:55	4	16
7	4	120	GS,MS	%ECP,CP,GS:MS ratio	8	6	50:50; 35:65	5	12
8	4	102	GS,MS	%ECP	6	1	45:55	1	17
9	4	72	GS,MS	%ECP	4	1	45:55	4-5	12
10	4	98	GS,MS	%ECP,CP	8	4	50:50; 55:45	5	12
11	2	60	GS,MS	DVE, GS:MS ratio	5	2	50:50	3	13
12	4	47	GS,MS	DVE	2	2	50:50	1	6
13	5	36	GS,MS	DVE	3	2	35:65	4	7
14	6	44	MS	DVE	4	4	65:35	3	20
15	6	43	MS	DVE	4	2	65:35	3	16
Total		905			70	30			

[a] Reference: 1: Rijpkema *et al.* (1990), 2: Rijpkema *et al.* (unpublished from IVVO-DLO, Lelystad), 3: Valk *et al.* (1990), 4: Veen *et al.* (unpublished from CLO-Institute for Animal Nutrition 'De Schothorst', Lelystad), 5: Boxem *et al.* (unpublished from Research Station for Cattle, Sheep and Horse Husbandry, Lelystad); 6: Hof *et al.* (1994)

[b] GS = grass silage, MS = maize silage, G = fresh grass;

[c] %ECP = escape protein fraction, CP = CP level, DVE = DVE level;

[d] Treatments with PU supply in at least one system below PU requirements;

[e] PP = pre-period, MP = main-period. The pre-period started directly after calving, except for experiment 4 (in lactation week 8) and experiment 5 (in week 14).

Table 2. Summary of the factors and coefficients used in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system

	System[a]							
	CP	DCP	PDI	AAT	AP	MP	AAS	DVE
Protein Unit requirements (g d^{-1})								
- Lactation								
- efficiency	0.38	0.52	0.64	0.73	0.65	0.72[b]	0.80	0.64
- Maintenance								
- metabolic faecal	-	-	-	-	0.09*IDM[c]	$1.43*W^{0.75}$	18.19*DM	[d]
- endogenous urine	-	-	-	-	$4.10*W^{0.5}$	$0.75*W^{0.75}$	[e]	$4.10*W^{0.5}$
- scurf	-	-	-	-	$0.30*W^{0.6}$	$0.11*W^{0.75}$	$0.11*W^{0.75}$	$0.30*W^{0.6}$
- total	[f]	[g]	$3.25*W^{0.75}$	$3.30*W^{0.75}$	-	-	-	-
- Body protein	-	-	-	-	-	[h]	-	[h]
Protein Unit supply (g kg^{-1} DM)								
Total	Table	Table						
- Microbial (M)								
- Energy unit	-	-	MOF[i]	DCH[j]	TDN[k]	FME[l]	ME[l]	FOM[m]
- MCP/energy unit	-	-	145	179	[n]	11	11.92	150
- MCP/degraded CP	-	-	0.90	1.00[j]	0.90	1.00	1.20	1.00
- M true protein/MCP	-	-	0.80	0.70	0.80	0.75	0.70	0.75
- MPU/M true protein	-	-	0.80	0.85	0.80	0.85	0.90	0.85
- Escape (E)								
- escape protein/CP	-	-	Table	Table	Table	Table	Table	Table[o]
- correction factor	-	-	1.11	-	-	-	-	1.11
- E true protein/ECP	-	-	1.00	0.65/0.85[p]	1.00	1.00	0.70	1.00
- EPU/E true protein	-	-	Table	0.82	0.80	Table	0.90	Table
- Correction factor	-	-	-	-	-	-	-15*DM[q]	-0.075*IDM[c,d]

[a] CP = Crude Protein; DCP = Digestible Crude Protein (CVB, 1990); PDI = Protein Digestible dans l'Intestin (Vérité, *et al.*, 1987); AAT = Aminosyres Absorbered fra Tyndtarmen (NKJ-NJF, 1985); AP = Absorbable Protein (NRC, 1985); MP = Metabolisable Protein (AFRC, 1992); AAS = Absorbierbare Amino Säure (Auschüss für Bedarfsnormen, 1986); DVE = DarmVerteerbaar Eiwit (Tamminga *et al.*, 1994).

[b] 0.72 = 0.68 (efficiency of true milk protein synthesis) / 0.95 (true protein content in milk CP)

[c] IDM (indigestible dry matter) = DM - digestible OM (DOM) - digestible ASH

[d] DVE-supply is corrected for metabolic faecal protein excretion

[e] endogenous urinary requirement for AAS: $(37.0*^{10}\log W - 42.2)$

[f] maintenance requirement for CP = (0.43*W + 130 - (600-W)*0.5)/0.7

[g] maintenance requirement for DCP = 0.43*W + 130 - (600-W)*0.5

[h] The PU requirement in the MP and DVE requirement for growth or supply from mobilisation of body reserves is dependent on energy balance:
for growth: 35 g DVE or 30 g MP/Mcal NEL positive energy balance
for mobilisation: 27 g DVE or 22 g MP/Mcal NEL negative energy balance

[i] MOF (fermentable organic matter, PDI-system) = DOM - escape CP - crude fat - fermentation products

[j] DCH (total digestible carbohydrates) = digestible crude fibre (DCF) + digestible non-fatty-extracts (DNFE);
200 g rumen degraded CP d^{-1} can be recycled to the rumen

[k] TDN (total digestible nutrients) = DOM + 1.25*digestible crude fat (DFAT)

[l] ME = metabolic energy calculated according to CVB (1991), FME = fermentable ME = ME - 35 * crude fat - 0.1 * ME (for silages) and ME - 35 * crude fat (for other feedstuffs)

[m] FOM (fermentable organic matter, DVE-system) = DOM - escape CP - crude fat - fermentation products * 0.5 - escape starch;

[n] microbial crude protein = (-31.86 + 26.12*(TDN/1000)) * 6.25

[o] escape CP/CP for grass, grass and grass silage is predicted from the CP and DM content and the number of days elapsed since the first of April (Tamminga *et al.*, 1994)

[p] true protein/CP roughages = 0.65; concentrates = 0.85

[q] correction for endogenous protein flow to the duodenum

Calculation of the energy and PU supply and requirement

Contents of dry matter (DM), organic matter (OM), CP, and Dutch Net Energy Lactation (NEL) were obtained from laboratory analysis of individual roughages, concentrates and concentrate ingredients. Lacking values were taken from the Dutch Feeding Tables (CVB, 1991). The fat content of grass, grass silage and maize silage was assumed to be 40, 40 and 30 g kg^{-1} DM, respectively.

The energy supply and requirements were calculated according to the Dutch NEL-system (Van Es, 1975, 1978). The following protein evaluation systems were used in the validation: CP and DCP-system (CVB, 1990), PDI-system (Vérité *et al.*, 1987), AAT-system (Madsen, 1985), AP-system (NRC, 1985), MP-system (AFRC, 1992), AAS-system (Ausschüss für Bedarfsnormen, 1986) and DVE-system (Tamminga *et al.*, 1994). The coefficients and factors used in each system to calculate PU supply and requirements are summarised in Table 2. In the AAS and DVE-system the sum of escape and microbial PU is diminished with respectively endogenous duodenal AAS and metabolic faecal DVE.

The protein escape fraction and digestibility of escape protein of the diets were calculated on the basis of the individual feedstuffs. Values for individual feedstuffs were taken from the tables of each system (AFRC, 1992; CVB, 1991; Hvelplund, 1985; Jarrige, 1990; Madsen and Hvelplund, 1985; NRC, 1988). When values were not available in those tables, data were obtained from the Dutch Feeding Tables (CVB, 1991), based on studies of Van Straalen and Tamminga (1990). The content of silage fermentation products in the PDI-system was taken from Jarrige (1990).

Potential microbial CP synthesis was calculated based on the rumen available energy and on the rumen degraded CP, both multiplied with the respective efficiencies, and for the AAT-system including recycling of degraded CP (Table 2). The lowest value in the diet was used as the actual microbial CP synthesis. The difference between microbial CP based on CP and based on energy was called the rumen protein balance (RPB).

For each system the PU available for milk production was calculated from total PU supply minus PU requirement for maintenance and for the MP and DVE-system also for retention or mobilisation of body protein. Maintenance requirements in the DVE-system only consist of requirements for endogenous urinary-N and skin-N, because metabolic faecal requirements were already substracted from the DVE supply. Milk protein production was predicted from the PU available for milk production and the efficiency of milk protein production. The absolute prediction error of milk protein production (APE) was calculated as the difference between predicted and observed milk protein production. NEL and PU supply, PU requirements and APE were calculated per cow and lactation week. Feed intake, milk production, NEL and PU supply and requirements and APE were averaged per treatment, resulting in 70 observations for further calculations.

Validation and statistical analysis

The validation of protein evaluation systems was carried out using a restricted database in which only treatments were included in which PU supply may have been limiting for milk protein production. Selected treatments therefore had to meet the condition that total PU supply was in at least one system below total PU requirements. This restricted the database to 30 treatments (Table 1), of which respectively 21 treatments were deficient in CP, 23 in DCP, 18 in PDI, 1 in AAT, 19 in AP, 0 in MP, 1 in AAS, and 16 in DVE. Differences between systems in PU supply, PU requirements and APE were tested using analysis of variance.

Validation of the systems was based on the following criteria:

1. The relationship between PU (and NEL) intake and milk protein production, using regression analysis;
2. The difference between predicted and observed milk protein production for each system, calculated as the mean square prediction error (MSPE), according to Bibby and Toutenberg (1977):

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

in which O_i and P_i is the observed and predicted milk protein production, and n is the number of observations (30). The MSPE was decomposed into the error due to overall bias (intercept different from 0), the error due to deviations of the regression slope from 1 and the error due to disturbances (Bibby and Toutenburg, 1977). The root of the MSPE expressed as percentage of the observed mean is used as measure of the relative prediction error (RPE).

The relationships between APE and feed intake variables (RPB, escape PU, microbial PU, NEL, NEL/PU for milk production), were studied by calculating correlation coefficients. These relationships were used to identify possible causes of the prediction error.

Statistical analysis was carried out using the Genstat Statistical Program (Genstat 5 Committee, 1987). Differences were declared at $p < 0.05$, unless stated otherwise.

Results

Milk production, feed intake, PU supply and PU requirements

Live weight, milk production and intake of DM, NEL and PU of the selected observations are summarized in Table 3. Milk protein production was on average 989 g

d^{-1} and ranged from 812 to 1271 g d^{-1}. PU intake in the new systems was highest for the AP-system (65% of CP) and decreased in the following order: MP (63% of CP), AAS and PDI (62% of CP), AAT (61% of CP) and DVE-system (54% of CP).

Table 3. Average, minimum, maximum and standard deviation (Std.) of live weight, milk production and DM, net energy lactation (NEL) and protein unit (PU)-intake (n=30)

	Average	Minimum	Maximum	Std.
Live weight (kg)	589	542	645	30
Milk production				
- FPCM[a] (kg d^{-1})	31.2	25.1	41.1	4.7
- protein (g kg^{-1})	32.3	30.4	34.4	1.0
- protein (g d^{-1})	989	812	1271	130
- fat (g kg^{-1})	41.9	31.8	48.0	3.8
- fat (g d^{-1})	1295	914	1747	236
DM-intake (kg d^{-1})				
- total	20.0	16.8	24.0	2.3
- roughage	10.9	5.9	16.2	2.9
- concentrate	9.0	3.4	11.6	2.5
- roughage (g kg^{-1} DM)	545	339	822	126
NEL-intake (MJ d^{-1})	133.3	107.0	164.7	18.4
PU intake (g d^{-1}) (between brackets: % of requirements)				
- CP	3084 (98)	2611 (90)	3871 (111)	340
- DCP	2195 (96)	1802 (85)	2772 (116)	248
- PDI	1904 (98)	1560 (84)	2425 (109)	240
- AAT	1886 (108)	1557 (99)	2382 (117)	231
- AP	2006 (97)	1678 (85)	2541 (108)	225
- MP	1952 (113)	1563 (103)	2419 (130)	216
- AAS	1907 (107)	1622 (98)	2410 (112)	219
- DVE	1664 (100)	1359 (89)	2077 (108)	240

[a] FPCM = fat and protein corrected milk
= [0.337 + 0.116*fat content (%) + 0.06*protein content (%)] * milk production (kg d^{-1})

PU supply from escape protein was different between systems, except for the PDI and AP-system, and varied from 21% of the sum of escape and microbial PU supply in the AAS to 46% in the DVE-system (Table 4). Microbial PU supply in the AAS-system was

Table 4. Average protein units (PU) supply and requirement, and predicted milk protein production in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system (g/d)

System[1]	PU-supply						PU-requirement		PU available for milk	Predicted milk protein production
	Escape[2]	Microbial[3] Energy	Microbial[3] CP	Microbial[3] Actual	Total	RPB[4]	Maintenance	Body		
CP	-	-	-	-	3084[a]	-	539[a]	-	2545[a]	967
DCP	-	-	-	-	2195[b]	-	377[c]	-	1818[b]	945
PDI	851[b]	1103[c]	1145[c]	1053[d]	1904[de]	66[c]	388[b]	-	1516[de]	970
AAT	713[d]	1240[d]	1275[b]	1173[b]	1886[e]	60[c]	394[b]	-	1492[de]	1101
AP	820[b]	1456[b]	1186[c]	1186[b]	2006[c]	-422[a]	541[a]	-	1465[e]	952
MP	778[c]	1330[c]	1182[c]	1173[b]	1952[cd]	-232[b]	274[d]	10	1667[c]	1194
AAS	448[e]	1687[a]	1793[a]	1648[a]	1907[de]	168[d]	546[a]	-	1362[f]	1089
DVE	921[a]	1123[c]	1240[b]	1100[c]	1664[f]	184[d]	113[e]	9	1542[d]	987
s.e.d.	37	39	47	36	65	68	10	-	61	-

[1] for abreviations see Table 2;
[2] figures with different superscripts in a column differ significantly ($p < 0.05$);
[3] Microbial PU supply based on rumen available energy, rumen degraded CP, and the actual microbial PU supply (average smallest microbial PU);
[4] Rumen protein balance.

highest compared to the other systems, for both methods of calculating microbial PU: from rumen available energy and from degraded CP. In the PDI-system microbial PU supply was lowest for both calculation methods. In the AP and MP-system, microbial PU supply from rumen degraded CP was lower than from rumen available energy, resulting in a high negative RPB for those systems. The actual microbial PU supply was highest for the AAS-system, and showed a small, although significant, variation among the other systems. The RPB was negative (microbial CP synthesis limited by rumen available protein) in 11, 12, 30, 25, 9 and 9 treatments of the 30 treatments for the PDI, AAT, AP, MP, AAS and DVE-system respectively.

PU requirements for maintenance were different between systems, except for the PDI and AAT-system, and the AP and AAS-system, and varied from 7% of the PU intake in the DVE-system to 29% in the AAS-system. The energy balance varied between -26 and 27 MJ NEL d^{-1} with an average of 0.4 MJ NEL d^{-1}. The correction for retention or mobilisation of body protein calculated from the NEL balance ranged from -152 to 207 g d^{-1} in the MP-system and -190 to 244 g d^{-1} in the DVE-system. The average was about 10 g MP or DVE d^{-1} which is 0.5% of the total MP or DVE requirement (Table 4). The PU available for milk production was highest in the MP-system. In the AAS-system, PU available for milk production was lowest compared to other systems. The predicted milk protein production varied from 945 g d^{-1} in the DCP-system to 1194 g d^{-1} in the MP-system.

Predictability of milk protein production

The relationship between milk protein production and total PU intake was highest for the AAT-system (R^2=0.82) and decreased in the following order: AAS (0.81), CP (0.79), AP (0.78), PDI (0.75), DCP and MP (0.71) and DVE (0.61). Total NEL intake showed a poor association with milk protein production (R^2=0.49). The relationship with milk protein production did not increase when PU and NEL intake were used in multiple regression.

The relationships between observed and predicted milk protein production are vizualised in Figure 1. Table 5 gives the APE, RPE and the decomposition of the MSPE. The average APE in the CP, DCP, PDI, AP and DVE-system was lower than in the AAT and AAS-system. The MP-system showed the highest APE. The RPE was lowest for the DVE-system and increased in the order of the CP, PDI, DCP, AP, AAS, AAT and MP-system. In the CP, DCP, PDI, AP and DVE-system most error was unexplained. Second highest source of error in the CP and DCP-system was due the difference of the intercept from 0, while in the PDI and DVE-system this was due to deviation of the regression slope from 1. In the AAT, MP and AAS-system most error was due to difference of the intercept from 0.

Table 5. Absolute and relative prediction error of milk protein production, and contributions of bias, regression and disturbances to the relative prediction error in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system

System[1]	Prediction error		Relative error due to		
	Absolute ($g\ d^{-1}$)	Relative (%)	Bias (%)	Regression (%)	Disturbance (%)
CP	-22	6.7	11.1	2.3	86.6
DCP	-44	8.8	25.6	4.4	70.0
PDI	-19	7.8	6.1	24.9	69.0
AAT	112	13.4	70.4	13.2	16.4
AP	-37	9.3	16.3	15.4	68.3
MP	204	22.9	81.0	8.5	10.5
AAS	100	11.7	74.8	4.1	21.1
DVE	-2	5.7	0.2	23.7	76.1

[1] for abbreviations see Table 2;

The most important correlation coefficients between the APE and feed intake variables are given in Table 6. The APE in the PDI, AAT and AAS-system were negatively related to the RPB. For all modern systems, APE was positively related to the escape PU intake and in the AAT and AAS system also to the microbial PU intake and NEL intake. The NEL-balance showed a relationship with the APE of all modern systems, except for the DVE-system. In all systems the APE had a close correlation between the PU available for milk production per NEL available for milk production.

Discussion

The escape protein fraction of feedstuffs was not measured in the experiments but were taken from tables that were published with the systems. This approach was preferred above using one escape fraction for each feedstuff whatever the system, because in a given system coefficients are based on the corresponding escape protein fraction (Waldo and Glenn, 1984). Only for some less important concentrate ingredients, escape fractions were lacking in some systems and were subsequently taken from CVB (1991).

The high actual microbial PU in the AAS-system, was the result of a high microbial PU supply from both rumen available energy and rumen available CP. The high microbial PU supply from rumen available CP was caused by the low escape fractions and high recycling capacity (20% of microbial CP synthesis) in this system. However, total AAS supply was comparable to the PU supply in other systems, because this high microbial PU supply was compensated by low escape PU supply. Because of high maintenance requirements, a relative small amount of the AAS supply was available for milk production, but this was compensated by the high efficiency of milk protein production, resulting in a predicted milk protein production comparable to other systems. Actual microbial PU supply in the AP-system was in all treatments limited by rumen degraded CP, and comparable to other systems. The AP-system therefore highly overestimates the microbial PU supply based on TDN in the diet, which was reflected in the highly negative RPB. To a lesser extend this also was true for the MP-system.

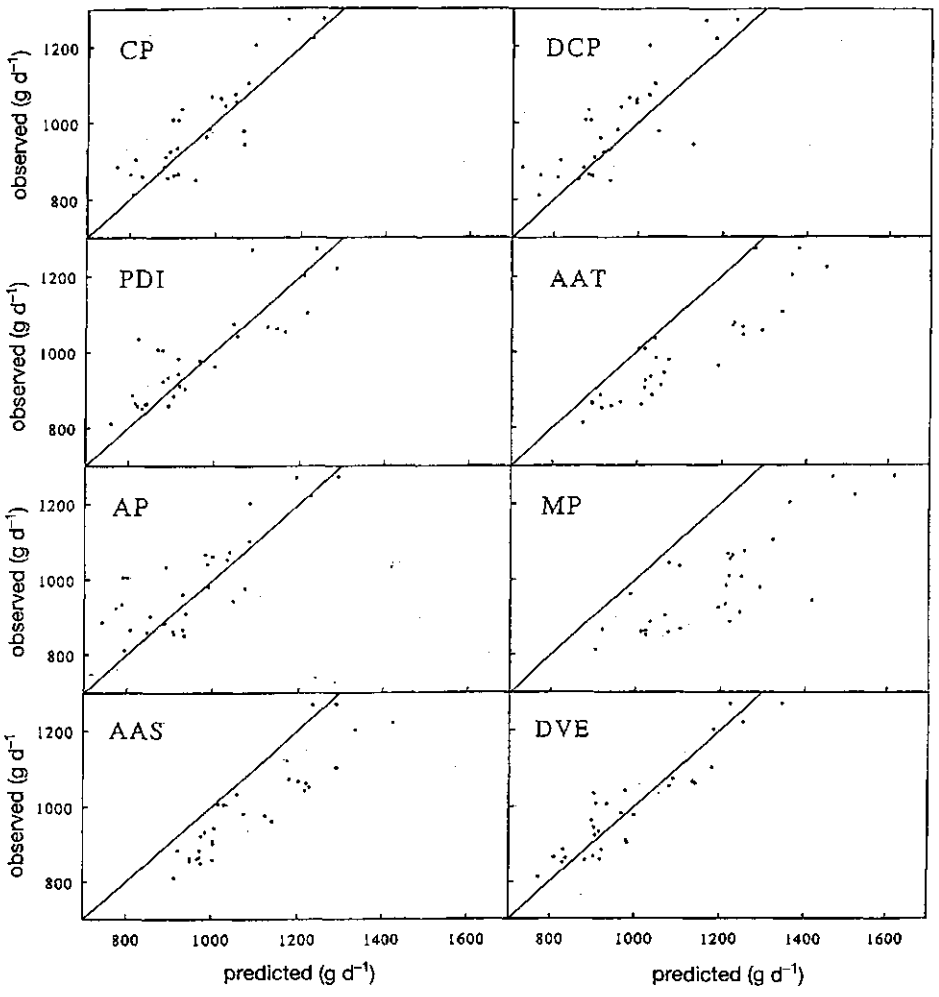
Table 6. Correlation coefficients between the absolute prediction error of milk protein production (APE) and the intake of rumen CP balance (g d^{-1}), escape PU (g d^{-1}), microbial PU (g d^{-1}), NEL (Mcal d^{-1}), NEL balance (Mcal d^{-1}) and PU/NEL available for milk production (g $Mcal^{-1}$) for the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system (n=30)

	Intake					
System[a]	Rumen CP Balance	Escape PU	Micro-bial-PU	NEL	NEL-balance	PU/NEL
CP	-	-	-	-0.11^{ns}	0.18^{ns}	0.82^{**}
DCP	-	-	-	-0.28^{ns}	-0.04^{ns}	0.86^{**}
PDI	-0.64^{**}	0.61^{**}	0.13^{ns}	0.42^{*}	0.51^{**}	0.84^{**}
AAT	-0.65^{**}	0.64^{**}	0.53^{**}	0.70^{**}	0.53^{**}	0.70^{**}
AP	0.15^{ns}	0.77^{**}	0.08^{ns}	0.11^{ns}	0.37^{*}	0.90^{**}
MP	0.32^{ns}	0.60^{**}	0.12^{ns}	-0.24^{ns}	-0.57^{**}	0.76^{**}
AAS	-0.52^{**}	0.40^{**}	0.37^{*}	0.54^{**}	0.74^{**}	0.69^{**}
DVE	-0.33^{ns}	0.65^{**}	0.03^{ns}	0.17^{ns}	0.14^{ns}	0.79^{**}

[a] for abbreviations see Table 2;

^{ns} = not significant; * = $p<0.05$; ** = $p<0.01$.

Figure 1. Relationships between observed and predicted milk protein production in the CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system (line indicates Y=X)



In the AAS and DVE-system, total PU supply was calculated from escape and microbial PU decreased with endogenous duodenal AAS (on average 188 g d^{-1}) or DVE required to compensate for metabolic faecal losses (on average 357 g d^{-1}). Adding the latter to the total PU intake resulted in values comparable to those in the other systems (2095 g AAS d^{-1} and 2021 g DVE d^{-1}). The average maintenance requirements were higher in systems where it was mainly based on feed intake (AP, AAS and DVE-system) than in systems where it was related to animal weight (PDI, AAT and MP-system). The low requirements in the MP-system compared to the other systems was caused by the high efficiency of MP use for maintenance purposes (1.00) compared to the other systems with a factorial approach of the maintenance requirements (0.67 in the AP and DVE-system).

High correlation coefficients between PU intake and milk protein production for the CP and DCP-system, comparable to the AAT-system, were also observed by Syrjälä-Qvist *et al.* (1985). However, Theun and Vik-Mo (1985) and Vik-Mo (1985) obtained better relationships with the AAT and PDI-system than with the DCP or CP-system. Variable results were obtained with the AP-system: Robinson *et al.* (1991) concluded that the NRC (1985) recommendations were too high, but Broderick *et al.* (1991) observed an increased milk protein production after increased AP intake. The AFRC (1992) reported, if variation between experiments was accounted for, a close association (R^2 = 0.94) between milk protein production and MP intake, which is in contrast to the poor results obtained with our dataset. According to AFRC (1992), MP was closely related to the PU calculated according to ARC (1984). Recommendations of the ARC (1984) were supported by Cody *et al.* (1991) and Robinson *et al.* (1991), but found to be too low by MacRae *et al.* (1988) and Vik-Mo (1985). The difference in performance within a system as reported in literature is hard to explain. Variable results might be caused by differences in feedstuffs and protein escape fractions used, energy and PU supply relative to requirements and milk production level.

In contrast with the MP-system, the correction of DVE supply for retention and mobilisation of body protein resulted in a better prediction of milk protein production. The DVE-system showed, in contrast to the other modern systems, no significant relationship between the APE and NEL-balance. For those systems including protein retention or mobilisation in the system might increase the predictability of milk protein production.

The higher APE in the AAT and AAS-system compared to the PDI and DVE-system could be explained by the higher efficiency of milk protein synthesis applied in the AAT and AAS-system. In the MP-system the high APE compared to the other systems was a combined effect of high MP intake, low MP maintenance requirement and high efficiency of milk protein production. In all modern systems the APE showed a close relationship with escape feed PU, from which can be concluded that variation in escape PU supply has less influence on milk protein production than expected. This can be due to overestimation of the escape fraction compared to *in vivo* data. Another explanation would

be that the amino acid profile of the escape protein was not in balance with the profile of amino acids needed for milk protein production.

In all systems a close positive relationship was observed between the APE and the ratio of PU/NEL available for milk production. The APE increased with increasing PU/NEL, which suggests that at higher PU intakes the efficiency at which PU is used for milk protein production decreases and illustrates the importance of the interaction between protein and energy at metabolic level (MacRae *et al.*, 1988). Part of this decrease could be explained by the fact that in the NEL-system the NEL requirement per kg of fat and protein corrected milk (FPCM) increases with milk production:

NEL for milk in MJ = 3.04*FPCM + 0.005*FPCM^2.

In contrast to the NEL-system, protein evaluation systems assume a constant efficiency. The observed efficiency could be calculated by dividing milk protein production by PU available for milk production. As with the APE, the observed efficiency showed a close relationship with PU/NEL available for milk production. Additional variation in observed efficiency could be explained by including fat protein corrected milk production (FPCM) in the equation. For the DVE-system the following equation was developed (R^2 = 0.75, n=30):

$$\underset{(g\ g^{-1})}{\text{efficiency}} = 1.22 - \underset{(g\ MJ^{-1})}{0.031*DVE/NEL} - \underset{(kg\ d^{-1})}{0.0032*FPCM} \quad (1)$$

The decrease in efficiency agrees with the commonly accepted diminishing returns with increasing protein intake (MacRae et at, 1988). With equation (1) the efficiency was predicted for the treatments in the original dataset that were not used for the validation. Despite the fact that all those treatments were overfed in DVE, the predicted efficiency (range 0.43 to 0.69) showed a close relationship with the observed efficiency (range 0.42 to 0.63) (R^2=0.84, n=40).

Milk protein production can be calculated from the DVE supply for milk production multiplied with the efficiency. Using equation (1), milk protein production can be calculated according to:

$$\text{milk protein} = (1.22 - 0.031*DVE/NEL - 0.0032*FPCM) * DVE \quad (2)$$

The DVE/NEL ratio for milk production, at which maximum milk protein is achieved, can be calculated by putting the first derivative of equation (2) equal to 0, which results in:

$$\frac{DVE}{NEL} = \frac{1.22 - 0.0032 * FPCM}{0.031 * 2} \quad (3)$$

With equation (3) it can be shown that DVE to NEL ratio at maximum milk protein production decreases from 19.2 at 10 kg FPCM d^{-1} to 17.6 at 40 kg FPCM d^{-1}, from which can be concluded that the increase in requirements with increasing FPCM production level is less pronounced for DVE than for NEL.

Conclusions

From the previous discussion it can be concluded that the prediction of milk protein production under Dutch conditions decreased in order of the DVE, CP, PDI, DCP, AP, AAS, AAT and MP-system. Predictions can be improved when a variable efficiency of milk protein production is used. In the DVE-system the observed efficiency decreased with increased protein to energy ratio in the diet and milk production level.

Acknowledgements

The authors are grateful to S. Tamminga of the Agricultural University and A.M. van Vuuren of the IVVO-DLO for their contribution to the discussion and critically reading the manuscript. This study was carried out at IVVO-DLO and partially financed by the Fund for Manure and Ammonia Research (FOMA).

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CHAPTER 4

RUMEN DEGRADATION AND INTESTINAL DIGESTION OF GRASS AND CLOVER AT TWO MATURITY LEVELS DURING THE SEASON IN DAIRY COWS

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Published in:
Grass and Forage Science (1994) 49, 378-390

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Abstract

A comparison was made of the effect of season and maturity on the in situ ruminal breakdown and intestinal protein digestion in dairy cows between intensively N-fertilized grass (whole sward), and moderately N-fertilized ryegrass and clover from a mixed sward. From May to September 1990, eight consecutive cuts were made, representing alternate harvests of late and early swards. Limited effects of N-fertilization on chemical and ruminal degradation characteristics and intestinal digestion of grasses were observed. Clover had higher ash, crude protein (CP) and lignin, and lower hemicellulose, cellulose and sugar contents compared to grass. Moreover, soluble fractions of organic matter (OM) and CP were higher, and rumen-undegradable OM and CP fractions were lower. Soluble fractions of grasses and clover decreased and undegradable fractions increased during the season. More mature swards showed increased undegradable fractions. Degradation rates of OM and CP in clover were, respectively, higher than or similar to those of grass. Seasonal effects on degradation rates differed between grass and clover. Effectively rumen degradable (g kg^{-1} DM) carbohydrates and CP, escaped protein and intestinal digestion of escaped protein were higher in clover than in grasses. Inclusion of clover in swards may result in higher post-rumen protein supply. Supplementation of clover based diets is suggested, to compensate for an increased loss of N in the rumen.

Introduction

Intensive husbandry in The Netherlands has resulted in the application of high levels of fertilizer-N on grassland. Under these circumstances perennial ryegrass has become the predominant grass species in most Dutch permanent pastures. These swards produce highly digestible roughage for dairy cattle. The recent trends are towards a reduction in fertilizer-N is expected to continue in the near future. Quota restrictions on milk production have reduced animal stocking per hectare. There is also a growing concern about the environmental consequences arising from excretions of nitrogen by cattle consuming large volumes of grass products with a high N content (Tamminga, 1992). Extensification, or a reduction in inputs, could provide part of the solution to these problems.

A reduction in fertilizer-N will promote the (re-)establishment of clover in swards, in particular on sandy and clay soils. Compared to intensively fertilized grass, grass/clover

pastures can reduce the loss of N by leaching (Sheldrick *et al.*, 1987). An increased presence of clover in the pastures may positively affect DM intake from the sward and enhance animal performance (Thomson, 1984; Bax and Thomas, 1992) as a result of a more rapid breakdown of clover OM in the rumen (Beever *et al.*, 1986). To establish an optimal presence of clover in the forage with respect to utilization of N and its excretion, quantitative information is needed on the extent of digestion of CP and non-protein OM in the rumen as well as in the intestines (Van Vuuren *et al.*, 1990). Beever *et al.* (1986) presented evidence for an increased microbial CP synthesis on clover. However, no experiments were performed studying grass and clover from mixed swards and simultaneously comparing them with ryegrass intensively fertilized with N.

In this study the influence of season and maturity on the *in situ* rumen degradation and post-rumen digestion of intensively N-fertilized grass, and of grass and clover simultaneously cut from a moderately fertilized grass/clover sward was investigated.

Materials and methods

Forages

Samples were prepared from swards originating from two plots on clay soil at the Waiboerhoeve Experimental Farm in Lelystad, sown in August 1988 with a *Lolium perenne* (cv. Profit/Magella) based permanent grassland mixture (Plot 1) and with *Trifolium repens* (cv. Retor) added to the same mixture (Plot 2). Nitrogen fertilizer application to Plot 1 and 2 was about 300 and 100 kg ha^{-1} respectively in the first harvest year preceding the experiment. In early spring 1990 the plots were fertilized with 106 and 91 kg N ha^{-1} (half organic origin) respectively. Additional applications of N to Plot 1 followed after each cut (1- 5) to a grand total of 315 kg N ha^{-1}. No additional fertilizer was applied to Plot 2. From early May to early September 1990, eight consecutive cuts from ten strips of *c.* 12 m^2 were made from both plots using a 'Haldrup' motor mower. Cutting dates were chosen to represent alternate harvests of a 'late' cut (> 3 t DM ha^{-1}) as is usual when harvesting for silage making and an 'early' cut (about 2 t DM ha^{-1}) as for grazing. The first cut was harvested at an advanced stage of maturity. The second, third and fourth cuts were harvested at an early and a late stage. After harvesting the early cut, the pasture was grazed by cows for 2 d. Parts of the pasture were protected against grazing and later harvested at a late stage of maturity. Regrowth started directly after grazing, residual material was not removed except after the fourth cut. The fifth cut was only harvested at an early stage of maturity. Information concerning the harvested material is given in Table 1. Immediately after cutting a representative subsample of the mixed sward (Plot 2) was manually separated in ryegrass and clover components to provide sufficient quantities (about 10 kg) of both forages for further studies. Ten subsamples (*c.* 1 kg each) were taken from Plot 1 (whole sward) and from perennial ryegrass and clover from Plot 2, and were stored at -40°C.

Sample preparation for rumen incubations

The frozen forage samples were chopped with a paper guillotine to lengths of *c.* 1 cm. After thorough mixing, sub samples corresponding to 5 g DM each were transferred into coded nylon bags (polyamide, pore size 41 μm, porosity 30%, Nybolt, Zurich, Switzerland), with an inner size of 9 x 18 cm. These were then closed with a plastic bundling strip and kept at -20° C until rumen incubation. During weighing triplicate sub samples were taken of each feed for laboratory analyses.

Animal experiments

Samples from the first four harvests were rumen incubated in July 1990 in four Holstein Frisian dairy cows, fitted with a rumen cannula (10 cm i.d., Bar Diamond Inc., Idaho, USA), grazing on a permanent pasture (85-90% perennial ryegrass) at the ID-DLO Experimental Farm. Additional daily concentrate allowance was 7 kg. Samples from harvests 5-8 were rumen incubated in the same animals grazing a similar pasture in September 1990. Daily concentrate allowance was reduced in accordance with milk production. By this time one animal had been dried off. For the determination of intestinal digestion four dairy cows, fitted with a T-shaped cannula at the proximal ileum, were used. At the time of the experiment (October 1990) three of the animals had been dried off. The stall-fed animals received 8-16 kg (depending upon milk production and condition score) of a mix of 0.4 artificially dried grass and 0.6 concentrates (DM basis).

Rumen incubations

Nylon bags containing the frozen forage samples were exposed to room temperature for 1 h preceding *in situ* rumen incubation for 3, 6, 12, 24, 48 or 336 hours. The number of bags of each feed incubated in each cow was 2, 2, 2, 3, 4 and 5 respectively for each incubation time. Incubation procedure was similar to Van Vuuren *et al.* (1989). One bag per feed per cow was reserved for zero incubation (blank). After rumen incubation the bags were rinsed under tap water and stored in ice until washing for 50 minutes with 60 l water at 10° C without spinning in a washing machine (Bosch V360), followed by oven drying at 70° C for at least 24 h. After weighing, feed residues were ground to pass a 1 mm sieve and pooled per feed, cow and incubation time.

Intestinal digestibility

The intestinal digestibiliy of rumen escape forage protein was measured by the mobile nylon bag technique. Forage samples were prepared and weighed into nylon bags as described above. Rumen incubation lasted 12 h. After washing, residues were freeze dried and ground to pass a 3-mm screen. Nylon bags (3x6 cm, polyamide, qualified as above) were

filled with 0.5 g DM rumen residue. After pepsin-HCl incubation (1 g pepsin, Merck, Darmstadt, Germany, 2000 FIP-U g^{-1}, digestion capacity NF XII = 1:10.000, in 1 l of 0.1 M HCL) for 1 h at 37° C, bags were introduced through the cannula into the duodenum and periodically recovered from the faeces (every 2 h). The recovered bags were stored at -20° C until all the bags had been retrieved. After washing in a domestic washing machine, residues were dried at 70° C and ground to pass a 1-mm sieve before analysis. Details of the techniques are given by Van Straalen *et al.* (1993).

Laboratory analyses

Laboratory samples of forages were prepared by grinding to pass a 1- mm sieve after freeze drying. Feed samples as well as incubation residues were analyzed for DM, ash and Kjehdahl-N according to standard procedures at ID-DLO (Steg *et al.*, 1990, Van Vuuren *et al.*, 1993). Neutral detergent fibre (NDF) analysis was done according to Robertson en Van Soest (1981). Feed samples were additionally analyzed for sugar (Steg *et al.*, 1990), acid detergent fibre (ADF) following neutral detergent pretreatment, acid detergent-insoluble N (ADIN) (Robertson and Van Soest, 1981) and acid detergent lignin (ADL) (Van Soest, 1967). ADF content was determined in incubation residues from one cow. OM digestibility of feed samples was determined *in vitro* with rumen fluid followed by pepsin-HCl according to a standardized procedure including *in vivo* tested grass samples in each run as described by Steg *et al.* (1990).

The content of non-structural carbohydrates (NCSs) in forages and incubation residues was calculated as: NSCs = OM - CP - NDF - crude fat. The content of crude fat was assumed to be 40 g kg^{-1} DM.

Calculation model for rumen degradability and intestinal digestibility

The forage OM, CP, NDF and NSC were assumed to be distinguishable into three fractions: the initial wash (S) fraction estimated from the nylon bags during washing without rumen incubation (zero incubation time), the rumen undegradable fraction estimated after 336 h incubation (U; Robinson *et al.*, 1986) and the slowly degradable fraction (D = 1 - S - U). The S-fraction of NDF was assumed to be zero. The rate of degradation of the D-fraction (k_d, per hour) was calculated according to a first-order degradation model, including a test for a lag phase preceding the onset of rumen degradation (Van Vuuren *et al.*, 1991). Degradation characteristics (S, U, D and k_d) of NSCs were calculated from those of OM, CP, NDF and crude fat, assuming that the degradation characteristics of OM and crude fat are similar.

Effectively fermentable fractions of protein and carbohydrates were calculated as the sum of the washable fractions and slowly rumen-degradable fraction. The latter fractions were calculated according to Ørskov and McDonald (1979), assuming a passage rate (k_p) of 0.045 h^{-1} for CP and NSCs (Van Straalen and Tamminga, 1990) and of 0.020 h^{-1} for NDF (Van

Vuuren *et al.* 1993a, b):

$$RD_i = D_i \times k_{di}/(k_{di} + k_{pi}), \quad (i = \text{CP, NDF or NCS}).$$

S-fractions were assumed to be directly and completely degraded.

The small intestinal protein digestibility of the rumen escaped feed protein was calculated as the disappearance of CP from mobile nylon bags after passage though the intestines and subsequent washing.

Statistical analyses

Rumen degradation data were analyzed using analysis of variance. Factors in the model were experiment, cow, combination of forage type and maturity and season. The combination of forage and maturity consisted of all combinations of forage type (whole sward, ryegrass and clover) and maturity level (late and early cut). Season was separated into four periods, each period containing consecutive cuts of a heavy and a light sward. Because intestinal digestion was measured in one experiment, *E* (experimental period) was removed from the model when testing for intestinal digestibility. The model can be described as follows:

$$Y_{ijkl} = \mu + E_i + C_j + (ExC)_{ij} + FM_k + S_l + (FMxS)_{kl} + e_{ijkl}$$

Where: Y_{ijkl} is the response variable; μ is the mean; E_i is the experiment effect ($i = 1, 2$); C_j is the cow effect ($j = 1..4$); $(ExC)_{ij}$ is the interaction between experiment and cow; FM_k is the combined forage and maturity effect ($k = 1..6$); S_l is the seasonal effect ($l = 1..4$); $(FMxS)_{kl}$ is the interaction between combined forage and maturity and season; e_{ijkl} is the unexplained residual error, assumed to be normally and independently distributed.

The combined forage and maturity effect was separated into five orthogonal contrasts: (1) grass *vs.* clover, (2) whole sward *vs.* ryegrass and (3), (4) and (5) late *vs.* early cut for whole sward, ryegrass and clover respectively. Significance of effects were declared at $P<0.05$. Statistical analysis was conducted using Genstat software (Genstat 5 Committee, 1987).

Results

Swards

General characteristics of the forages harvested are given in Table 1. Owing to extremely favourable weather conditions for grass growth in spring 1990, more than 6 t DM ha^{-1} was harvested at the first cut from both plots in early May. Yields from other cuts varied according to the cutting regime. Except for the first cut, DM-production from Plot 1 was always higher than from Plot 2, ranging from 0.06 by the end of June to 0.65 in mid and late

Table 1. General characteristics of forages harvested

Harvest Date	Cut[1] E	Cut[1] L	Days regrowth	Plot 1 Code[2]	Plot 1 Yield (kg ha^{-1}) DM	Plot 1 Yield (kg ha^{-1}) N	Plot 2 Code[2]	Plot 2 Yield (kg ha^{-1}) DM	Plot 2 Yield (kg ha^{-1}) N	Proportion of clover DM	Proportion of clover N
8 May		1	-	WL1	6130	157	RL1/CL1	6169	171	0.14	0.20
5 June	2	↓	28	WE1	2430	67	RE2/CE2	1666	52	0.24	0.35
20 June	↓	2	42	WL2	4795	104	RL2/CL2	4051	107	0.27	0.40
26 June	3		21	WE3	2742	80	RE3/CE3	2579	70	0.23	0.34
10 July	↓	3	35	WL3	4410	102	RL3/CL3	3466	92	0.23	0.34
17 July	4		21	WE4	2820	75	RE4/CL4	1708	46	0.17	0.26
31 July		4	35	WL4	4011	92	RL4/CL4	2430	65	0.29	0.40
4/11 September	5		34	WE5	2783	75	RE5/CE5	1891	56	0.15	0.20

[1] L, late cut; E, early cut; arrows indicate regrowth

[2] W, Whole sward; R, Ryegrass; C, Clover; number refers to cut number

July. Yield of N from Plot 1 was also higher (0.11 to 0.63) than from Plot 2, except for the first two heavy cuts.

The botanical composition of both plots was determined before and after the experiments, on 19 October 1989 and 5 October 1990. According to botanical analysis 0.95 and 0.93 respectively of the sward from Plot 1 was *Lolium perenne*, with small amounts of *Poa annua* and *Poa trivialis*. The presence of *Lolium perenne* in Plot 2 was 0.68 and 0.73 respectively and of *Trifolium repens* was 0.28 and 0.22. Small amounts of *Poa annua* and *Poa trivialis* were found in October 1990. Separation of the crops from Plot 2 was always satisfactorily completed within 4 h after cutting. The DM content of samples taken at harvest and after separation showed no significant influence of this treatment, demonstrating that evaporation losses were minimal.

Chemical composition

No large differences in chemical composition between whole sward and ryegrass were observed (Table 2). Because of dry weather conditions and possibly residual grass from previous grazings, the DM contents of the samples from forages harvested on 17 and 31 July were distinctly above average. The CP content in clover was higher than in grasses. The CP content in ryegrass showed less variation than in whole sward. The proportion of ADIN in total N (g ADIN kg^{-1} N) was particularly affected by season; differences between grasses and clover were small, and the late cut from ryegrass and clover had higher averages than the early cut. Also ADIN in ADF (g ADIN kg^{-1} ADF) increased during the season. Clover had higher values than grasses; differences between maturity levels were small. No important effect of sward maturity was observed on NDF. However, a linear increase due to season was evident with a slight interaction with maturity. In contrast to NDF, ADF content was affected by maturity. ADL content increased linearly during the season. Proportional contribution of hemicellulose (calculated as NDF-ADF) was on average ($\pm$ standard deviation: s.d.) 0.50 ± 0.03 of total NDF in both whole sward and ryegrass versus 0.38 ± 0.06 in clover. Hemicellulose content of grasses and clover increased during the season, but the increase was greater in clover. Cellulose content (calculated as ADF-ADL) showed little variation with 234 ± 14, 238 ± 14 and 152 ± 13 g kg^{-1} DM for whole sward, ryegrass and clover respectively. The NSC content was on average 185 ± 41, 178 ± 44 and 286 ± 35 g kg^{-1} DM for whole sward, ryegrass and clover respectively. Within gras the NSC content was lower than that of the early cut grass. A marked decrease in NSCs was observed during the season.

Table 2. Chemical composition of forages (g kg^{-1} DM)

Code[1]	DM (g kg^{-1})	Ash	CP	NDF	ADF	ADL	Sugar	ADIN (g kg^{-1}) N	ADF	OMD (%)
Whole sward (Plot 1)										
WL1	165	107	160	470	247	14	127	20	2.0	81.4
WE2	151	120	173	427	220	12	136	17	2.1	81.2
WL2	146	106	136	494	272	18	124	26	2.1	77.0
WE3	167	115	182	484	256	17	89	32	3.6	75.3
WL3	162	105	145	549	274	25	103	50	4.3	73.8
WE4	233	113	167	531	252	22	107	43	4.6	74.0
WL4	240	104	144	521	247	23	127	51	4.8	72.5
WE5	160	112	168	568	262	25	77	58	6.0	69.9
Ryegrass (Plot 2)										
RL1	160	113	161	478	251	13	114	21	2.1	83.3
RE2	174	125	168	444	227	15	123	19	2.2	80.5
RL2	147	119	135	494	270	18	114	45	3.6	76.7
RE3	182	118	141	494	258	17	116	40	3.5	77.7
RL3	163	117	143	540	281	24	95	58	4.7	75.4
RE4	246	124	151	520	256	27	99	48	4.5	73.9
RL4	237	120	143	521	255	25	105	60	5.4	73.2
RE5	150	126	178	578	268	27	48	54	5.7	69.7
Clover (Plot 2)										
CL1	124	137	246	268	175	23	44	37	8.3	81.3
CE2	134	127	275	220	148	21	31	21	6.2	81.2
CL2	112	129	244	263	181	24	39	44	9.6	78.7
CE3	121	129	264	260	175	25	28	43	10.3	78.9
CL3	117	122	240	345	204	43	45	55	10.3	76.4
CE4	177	120	259	339	180	38	34	51	11.8	76.1
CL4	183	110	228	370	215	53	36	56	9.5	71.7
CE5	152	113	232	354	200	36	31	46	8.6	73.6

[1] For explanation see Table 1.

[2] OMD, *in vitro* digestibility of organic matter.

Rumen degradability

One animal showed overall a more complete and faster digestion (lower U fractions and higher k_d) than the other three cows, in particular for the clover samples harvested in the second half of the season. Data from this cow were not excluded; statistical analysis revealed that conclusions on main effects were not influenced by exclusion.

As the inclusion of a lag phase preceding degradation did not improve the fit, rates of degradation given were derived without considering a lag phase. Average degradation characteristics of OM, CP and NDF are given in Table 3, Figures 1-3 show their seasonal variation.

Organic matter

The initial wash fraction of OM was higher in clover than in grass samples (Table 3); both declined as the season progressed (Figure 1, S). In the last two late cuts, this fraction was considerably higher in the whole sward than in ryegrass. The undegradable fraction of OM was lower in clover than in grasses. No difference due to maturity was observed in whole sward and ryegrass, while the U-fraction in late-cut clover was higher than in the early cut. For all forages, undegradability increased as the growing season progressed (Figure 1, U). Seasonal variation was similar for maturity levels within each forage and for whole sward and ryegrass (Table 3). The potentially degradable OM fraction was lower in clover than in grass, and was mainly affected by sward maturity. Variation in this fraction was generally limited (s.e. 0.024 and 0.030 g g^{-1} OM for ryegrass and clover respectively). The degradation rate of clover OM was faster than for grass, with an average difference of almost 0.02 g g^{-1} h^{-1}. The difference was largest in mid-season, when a decrease was observed for the grasses, but not for clover (Figure 1, k_d).

Crude protein

The average initial wash fraction of CP was higher in clover than in grass (0.034 g g^{-1} CP), but with a large variation. This fraction was higher with more mature swards (Table 3) and a decrease during the season was evident for clover but less clear for grasses (Figure 2, S). Average rumen-undegradable fraction of CP was 0.070 g g^{-1} CP higher in grass than in clover, but when expressed as a proportion of DM it was almost identical in clover and ryegrass (averages 25 and 26 g kg^{-1} DM respectively). The late-cuts of all forages had a higher undegradable protein fraction than the early cut. An increase was observed during the season (Figure 2, U), with different patterns between forages and maturity levels (Table 3). The average potentially degradable fraction of CP was 0.037 g g^{-1} CP higher in clover than in grass. The late-cut showed lower values than the early cut for all forages. As shown in Figure 2 (k_d), relatively large differences in the degradation rate of CP between grass and clover were observed for the first two cuts. For both grasses and clover overall variation in degradation rate was rather limited (s.e. 0.011 g g^{-1} h^{-1}).

Table 3. Means of rumen degradation characteristics of organic matter, crude protein and neutral detergent fibre, and small intestinal digestibility of rumen escaped protein (SD_{EP}) and statistical significance of differences

Forage	Organic matter				Crude protein				Neutral detergent fibre			
	S[1]	U[1]	D[1]	k_d[1]	S	U	D	k_d	U	D	k_d	SD_{EP}
Means[2] of roughage and maturity combination												
1. WE[3]	0.14	0.17^{a}	0.69^{b}	3.8^{b}	0.17	0.15^{b}	0.68^{b}	8.9^{a}	0.19^{c}	0.81^{a}	3.0^{b}	0.84^{c}
2. WL	0.17	0.18^{a}	0.66^{c}	3.3^{c}	0.18	0.19^{a}	0.62^{d}	7.5^{cd}	0.19^{c}	0.81^{a}	2.6^{c}	0.84^{c}
3. RE	0.13	0.17^{a}	0.70^{a}	3.8^{bc}	0.16	0.16^{b}	0.68^{b}	8.6^{ab}	0.18^{c}	0.82^{a}	2.9^{bc}	0.85^{bc}
4. RL	0.14	0.17^{a}	0.69^{ab}	3.4^{bc}	0.19	0.18^{a}	0.63^{d}	8.2^{abc}	0.19^{c}	0.81^{a}	2.7^{bc}	0.85^{b}
5. CE	0.20	0.12^{c}	0.68^{b}	5.7^{a}	0.18	0.09^{d}	0.73^{a}	7.1^{d}	0.22^{b}	0.78^{b}	4.5^{a}	0.92^{a}
6. CL	0.23	0.15^{b}	0.63^{d}	5.7^{a}	0.24	0.11^{c}	0.65^{c}	7.8^{bcd}	0.25^{a}	0.75^{c}	4.3^{a}	0.92^{a}
s.e.d.[4]		0.005	0.005	0.19		0.005	0.005	0.43	0.006	0.006	0.19	0.005
P-values of contrasts of Roughage and Maturity combination												
(1+2+3+4) vs (5+6)		<0.001	<0.001	<0.001		<0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.001
(1+2) vs (3+4)		0.449	<0.001	0.785		0.084	0.057	0.536	0.256	0.256	0.771	0.009
1 vs 2		0.361	<0.001	0.012		<0.001	<0.001	0.002	0.651	0.651	0.015	0.715
3 vs 4		0.544	0.174	0.079		<0.001	<0.001	0.329	0.830	0.830	0.251	0.249
5 vs 6		<0.001	<0.001	0.985		0.003	<0.001	0.119	<0.001	<0.001	0.224	0.362
P-values of contrasts of interactions between season and roughage and maturity combination												
(1+2+3+4) vs (5+6)		<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.002
(1+2) vs (3+4)		0.452	0.056	0.475		<0.001	<0.001	0.023	0.502	0.502	0.605	0.616
1 vs 2		0.551	<0.001	0.951		0.005	<0.001	0.050	0.067	0.067	0.750	0.035
3 vs 4		0.118	0.040	0.299		0.020	<0.001	0.067	0.398	0.398	0.512	0.001
5 vs 6		0.098	0.468	0.650		0.020	<0.001	0.250	<0.001	<0.001	0.310	0.984

[1] S, U and D: instantly washable, undegradable and slowly degradable fractions respectively; $k_d/100$=degradation rate h^{-1}.

[2] figures with different superscripts in the same column differ significantly ($P<0.05$).

[3] for codes see table 1.

[4] s.e.d. = standard error of difference.

Figure 1. Seasonal variation in average initial wash fraction (S), undegradable fraction (U) and degradation rate of slowly degradable fraction (k_d) of OM of early (□- - -□) and late (●-----●) cuts of whole sward, ryegrass and clover

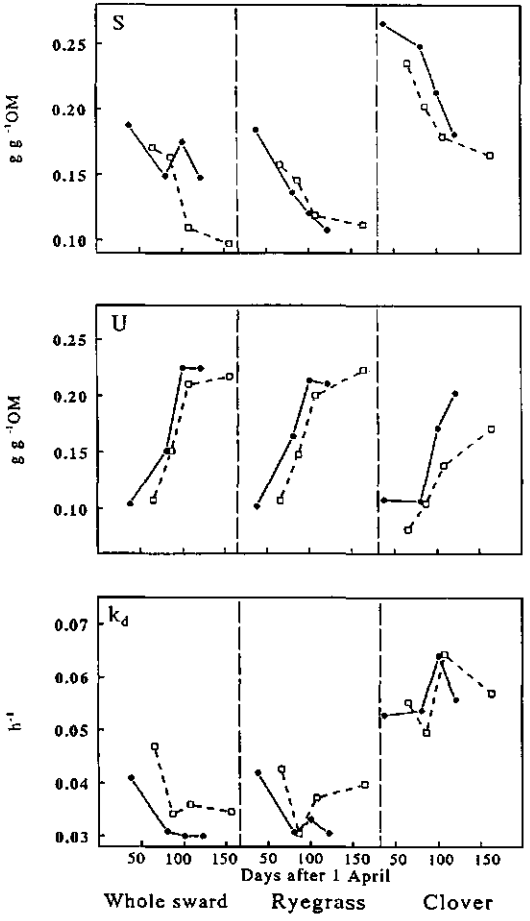
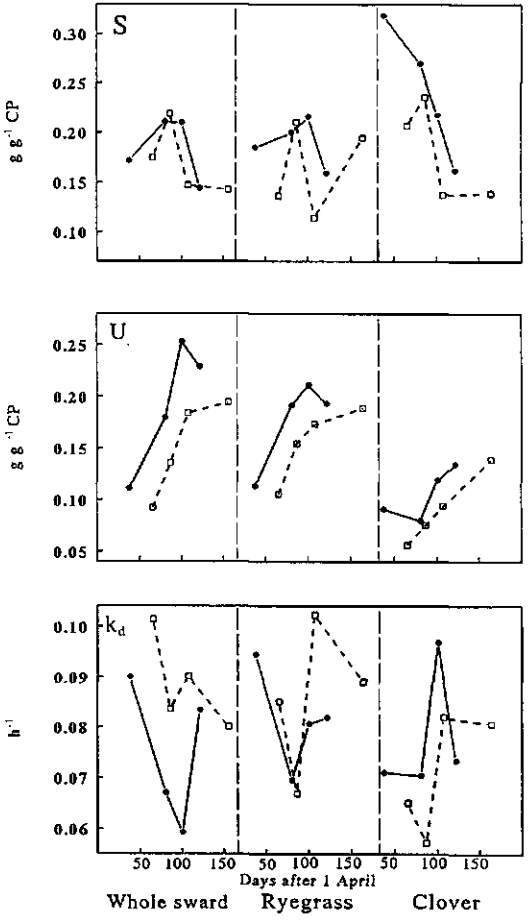


Figure 2. Seasonal variation in average initial wash fraction (S), undegradable fraction (U) and degradation rate of slowly degradable fraction (k_d) of CP of early (□- - -□) and late (●-----●) cuts of whole sward, ryegrass and clover



Carbohydrates

A problem was encountered in the derivation of rumen degradation characteristics of the cell walls. NDF contents measured in oven-dried zero-incubation samples were consistently higher than those found in comparable samples which had been freeze-dried. Additional analyses of the original material revealed that the difference was due to differences in drying procedure. Rumen degradation characteristics of NDF and ADF were calculated assuming that the washable fraction was equal to zero. The undegradable fraction of NDF was higher for clover than for grass and maturity had only a positive effect with clover (Table 3). An increase was observed during the season, with a different pattern between grass and clover and maturity levels of clover (Figure 3, U). As the potentially degradable fraction of NDF was calculated from the undegradable fraction, the statistical significances of differences were identical. Clover displayed a higher rate of degradation of NDF than grasses; the difference between maturity levels was only significant for ryegrass. Variation in degradation rate was small (s.e. 0.004 g g^{-1} h^{-1}) for all forages (Figure 3, k_d). The undegradable fraction of ADF was 0.020-0.040 g g^{-1} ADF higher than that of the NDF in both grass and clover; the degradation rate was only marginally lower for both species. Differences between grass and clover were significant and the undegraded fraction of ADF increased during the season.

The average calculated initial wash fractions of NSCs of whole sward, ryegrass and clover were 0.39, 0.36 and 0.46 g g^{-1} NSC, the undegradable fractions 0.19, 0.20 and 0.09 g g^{-1} NSC and the degradation rates of the slowly degradable fraction 0.067, 0.056 and 0.075 h^{-1} for whole sward, ryegrass and clover respectively.

Effectively rumen degradable protein and carbohydrates

Average data on effectively fermentable OM, initial wash value of CP and carbohydrates, slowly degradable protein and carbohydrates and their ratios are given in Table 4. Average effectively fermentable OM in clover was 82 g kg^{-1} DM higher than in grass. Less variation was found in clover (s.d. 30 and 49 g kg^{-1} for clover and grass respectively). The decline in fermentable OM during the season was much more pronounced for grass, mainly because of a decrease in washable carbohydrates. Effects from sward maturity were limited.

For grass, effectively degradable protein was negatively affected by maturity. Effectively degradable carbohydrates in grass showed considerable seasonal variation, from more than 400 g kg^{-1} DM for the first two harvests to slightly more than 300 g kg^{-1} for the last cuts. Variation was much more limited for clover and no clear seasonal or maturity effect was evident.

The ratio of the initial wash fraction of CP to carbohydrates of grass increased from 0.2 in May to very high values in September (2.0) in grass. Variation was smaller (0.27-0.58) in clover. The ratio of slowly degradable CP to carbohydrates was consistently higher for clover than for grass and higher for early cuts than for late cuts. For grass this ratio was lowest for late cuts in mid-season (0.16-0.22).

Figure 3. Seasonal variation in undegradable fraction (U) and degradation rate of slowly degradable fraction (k_d) of NDF of early (□- - -□) and late (●-----●) cuts of whole sward, ryegrass and clover

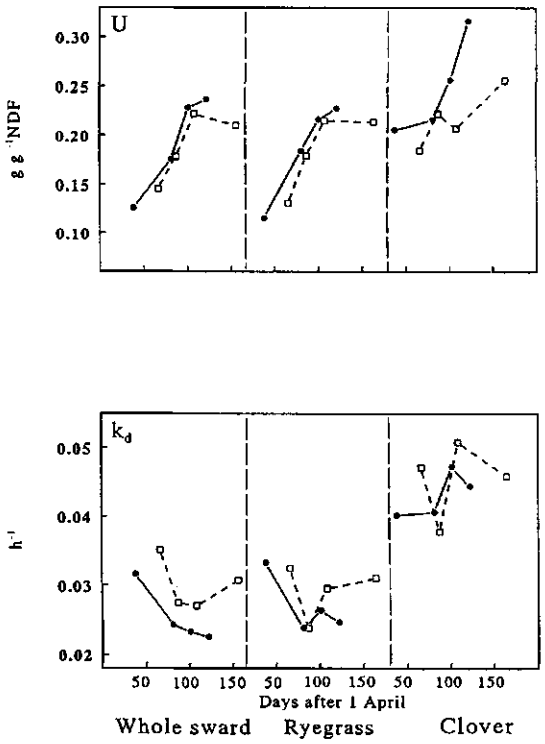
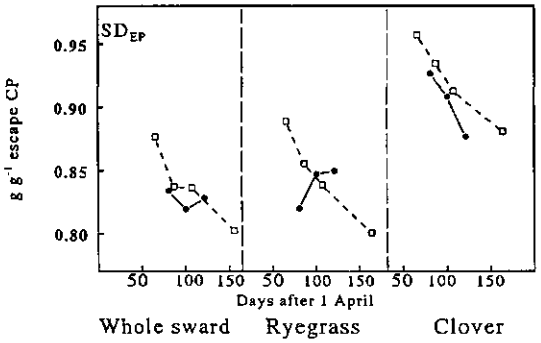


Figure 4. Seasonal variation in the intestinal digestion of rumen-escaped protein (SD_{EP}) of early (□- - -□) and late (●-----●) cuts of whole sward, ryegrass and clover



Intestinal digestibility

The digestibility of escaped protein in the small intestine was clearly higher in clover than in grass (Table 3). The stage of maturity did not have a significant effect. However, a declining influence of season was clearly observed for the early cuts of grass and for both cutting regimes of clover (Figure 4). As no observations on intestinal digestion were made for the first cut in May, the seasonal range was very limited for the late-cut samples.

Table 4. Averages of total fermentable organic matter (FOM), washable and slowly degraded crude protein (SCP, RDCP) and carbohydrates (SCB, RDCB) (g kg^{-1} DM) and their ratios

Forage	Maturity	FOM	SCP	SCB	RDCP	RDCB	Ratio SCP/SCB	Ratio RDCP/RDCB
Wole sward	Early	461	30	73	78	281	0.41	0.28
	Late	421	27	71	51	272	0.38	0.19
Ryegrass	Early	450	26	70	71	283	0.37	0.25
	Late	416	27	54	54	280	0.49	0.19
Clover	Early	540	47	148	114	230	0.31	0.50
	Late	519	52	118	102	248	0.44	0.41

Discussion

Swards

The fertilizing and cutting regime chosen does not allow for firm conclusions to be drawn on the comparative effect of fertilizer-N and clover presence on DM production. The lower DM yield observed for Plot 2 as compared to Plot 1 corresponds with data from the review of Bax and Thomas (1992). However, these authors observed surprisingly little effect on DM yield in first-cut silage (4 - 6 t DM ha^{-1}). The proportion of clover in the mixed sward from the current trial corresponds well with UK information (Bax and Thomas, 1992) and also data from Dutch conditions (Baars and Van Dongen, personal communication).

Chemical composition

No important differences in chemical composition (DM basis) were observed when

comparing the whole sward harvested from Plot 1 with the ryegrass samples selected from the sward from Plot 2. However, it should be considered that the sward from Plot 1 contained more than 90% perennial ryegrass. No noticeable effect was observed resulting from the selection process necessary to derive the ryegrass samples from Plot 2.

Owing to lower applications of fertilizer-N, ryegrass samples from the second and subsequent cuts were expected to have a higher DM, NDF and lower CP content than the whole sward. The lack of these differences is possibly the result of nitrogen fixation by the *Rhizobium* bacteria attached to the clover. According to Frame and Newbould (1984) 90-125 kg N ha^{-1} $year^{-1}$ may be fixed with proportionally 0.25 clover in the sward. Bax and Thomas (1992) reported up to 280 kg N fixation ha^{-1} $year^{-1}$ for clover-rich swards. CP contents in the grass samples were lower than the tabulated figures of 190-200 g CP kg^{-1} DM provided by CVB (1991) as an indication of Dutch swards fertilized with 300 kg N ha^{-1} yr^{-1} (at yields of 1.7-3 t DM ha^{-1}). The relatively low CP content may be partly due to weather conditions (Valk *et al.*, 1990). In addition, the swards tended to be more mature than expected. Sward maturity clearly affected CP content: the average difference between the early and the late-cuts was almost 30 g kg^{-1} DM. As expected, the CP content of clover was markedly higher than that of grass; the average level of 250 g CP kg^{-1} DM agrees with observations by Beever *et al.* (1986). The differences in CP content between early and late-cuts diminished during the growing season. When calculating the CP content of the mixed sward (Plot 2) from the proportional contribution of grass and clover, average CP content of the mixture was higher by 13 g CP kg^{-1} DM than the whole sward, whereas Bax and Thomas (1992) concluded that N-fertilized swards generally contained more CP. In our study, ADIN (g g^{-1} DM) was higher in clover than in grass. ADIN expressed as a fraction of total N showed little difference between grass and clover, and ADIN expressed as a fraction of ADF was higher in clover than in grass; both values increased during the season, as was also observed by Sanderson and Wedin (1989). However, the averages in our study were higher than those reported by Sanderson and Wedin (1989), which may be the result of difference in species, maturity level, or growing conditions.

The average NDF content of grasses was considerably higher than in the experiments of Van Vuuren *et al.* (1991, 1993a,b), Valk *et al.* (1990) and Ulyatt *et al.* (1988), indicating that in our experiments generally more mature grass was harvested. The NDF content of clover was on average almost 200 g kg^{-1} DM lower than that of grass, and the composition of the cell wall fractions of grass and clover differed greatly: averages of fractions recovered as hemicellulose, cellulose and ADL were 0.50/0.47/0.04 and 0.38/0.52/0.11 for grass and clover respectively. Much more seasonal variation in ratios was observed in clover than in grass. This was in line with the data of Thomson (1984). When expressed in g kg^{-1} DM both hemicellulose and cellulose were consistently lower for clover than for grass; lignin content was higher. The analytical procedure followed to determine ADF had a considerable effect on the calculation of the hemicellulose/cellulose ratio: samples from experiment 1 were also analyzed for ADF without neutral detergent pretreatment. Higher residues were determined as expected, but the difference was greater in clover (55 ± 7 g kg^{-1} DM) than in whole sward

and ryegrass samples (13±5 g kg^{-1} DM).

Average *in vitro* digestibility of the grass samples was found to be lower than in experiments carried out by Van Vuuren *et al.* (1991) and Valk *et al.* (1990), reflecting harvesting at a more mature stage. *In vitro* digestibility values tended to be higher for clover than for grass, inspite of a higher lignin content. Cutting clover at a more mature stage resulted in a similar drop in digestibility as with grass, with the largest effect being noticed in July. For both grass and clover a clear but different relationship was observed between *in vitro* digestibility and ADL content.

Rumen degradability

Organic matter

Differences in the initial wash value between whole sward and ryegrass may be partly explained by the difference in sugar content. Rate of degradation of the potentially degradable OM-fraction for the grass samples was lower than most data presented by Van Vuuren *et al.* (1991, 1993a,b), except for plots which were not fertilized.

The faster and more complete OM degradation in clover might be due to the lower cell wall fraction when compared to the grasses. However, despite a clear seasonal increase in cell wall content of clover and increasing lignification, no decrease in rate of OM degradation nor in NDF degradation was recorded. As yet, the reason for the absence of any effect remains unclear. The decrease observed during the growing season in wash fraction of OM in grass is in agreement with data of Beever *et al.* (1986). The level corresponds with that of the experiments of Van Vuuren *et al.* (1991), but was lower than reported by Van Vuuren *et al.* (1993a,b), who incubated freeze-dried and ground samples. Beever *et al.* (1986) observed much higher initial wash fractions for clover in two of the three experiments reported. Differences in sample preparation, pore size of the nylon bags used and washing procedure might be responsible. The rumen-undegradable OM fraction was observed to increase with season and maturity for both grass and clover. The OM fraction of grass not degraded in the rumen was larger than observed by Van Vuuren *et al.* (1991), and was most likely a reflection of harvesting at a more mature stage, as was also demonstrated by higher levels of cell wall characteristics and lower *in vitro* digestibility.

Crude protein

On average 0.13 to 0.22 g g^{-1} of the grass CP was instantly washed from nylon bags, with little effect of stage of maturity or cutting date. Van Vuuren *et al.* (1991) observed higher values for the wash fractions in a maturation trial (0.21-0.37) and lower values in fertilizer-N experiments (0.06-0.25) with very young grass. High values (0.40-0.53) were observed by Van Vuuren *et al.* (1993a,b) using freeze-dried and ground grass samples. It seems conceivable that this washable fraction decreases with maturity, but small differences in sample preparation procedures could complicate the comparison between experiments. The rumen-undegradable protein fraction of grass increased substantially during the growing

season and as a result of sward maturity. The values were also higher in this experiment (0.09-0.25) than reported by Van Vuuren *et al.* (1991, 1993a,b), with the exception of one plot which received no fertilizer-N, and higher than data from Beever *et al.* (1986). The undegradable fraction was also higher than predicted from CP content and cutting date in the equation given by Van Straalen and Tamminga (1990). One of the explanations for the differences is the maturity stage. It is conceivable that at lower protein contents a larger fraction is bound to cell walls and therefore contributes to a low degradability. However, a maximum of 0.06 g g^{-1} of the N in grass was insoluble in acid detergent, which would account for only 0.18-0.33 of the undegradable protein fraction. Maturation depressed protein degradation rate except for the ryegrass samples of the third cut. The seasonal effect was mainly linear as calculated by Van Straalen and Tamminga (1990). Van Vuuren *et al.* (1991, 1993a,b) showed similar rates of degradation for grass fertilized with 0-250 kg N ha^{-1} $year^{-1}$. They noticed higher degradation rates with N-applications in excess of 250 kg N ha^{-1}.

The fraction of CP in clover which was initially washed out of the nylon bags was considerably higher in the first two cuts, probably due to a larger non-protein nitrogen fraction. As a result, in agreement with observations of Beever *et al.* (1986), average values in clover samples were higher than in grass. The undegradable CP fraction of clover increased with season and maturity, but remained considerably lower than grass, despite the fact that the ADIN content in clover was higher than in grass.

Cell wall fractions

The undegradable fraction and degradation rate of NDF in grasses were higher and lower respectively than reported by Van Vuuren *et al.* (1993a,b), who incubated freeze dried and ground material. Data concerning ruminal breakdown of NDF showed that undegradable NDF in both grasses and clover constituted on average 0.62 of total undegradable OM, with little variation due to forage, maturity level or season (total range 0.57 to 0.68). After correction for undegradable NDF and CP, the undegradable OM also showed little variation: 15-42 g kg^{-1} DM for grass and 16-32 g kg^{-1} DM for clover. Neither season nor maturity affected this fraction considerably.

Effectively rumen-undegradable protein and carbohydrates

The assumptions made on fractional passage rates result in a slightly higher overall passage of OM from clover than from grass. The higher effectively fermentable OM content of clover compared with grass supports the observations of Beever *et al.* (1986) that potential for microbial synthesis is higher in clover than in grass. According to Van Vuuren *et al.* (1990), optimally 25 g N should be made available per kg OM fermented in the rumen. This implies a fermentable CP / fermentable OM ratio of 0.16. Ratios observed were always higher than 0.21, suggesting that N would not be limiting rumen fermentation or microbial synthesis. Van Vuuren *et al.* (1990) tested the same criteria as the ratio between fermentable CP and fermentable carbohydrates, which seems debatable as fermentable OM always

contains an amount of fermentable CP. Correction for this would imply an optimal ratio of about 0.19 for both the washable and the slowly degradable protein compared with carbohydrates. When using this criterion, rumen available N could have been limiting microbial activity on grass harvested at a late stage of maturity during mid-season.

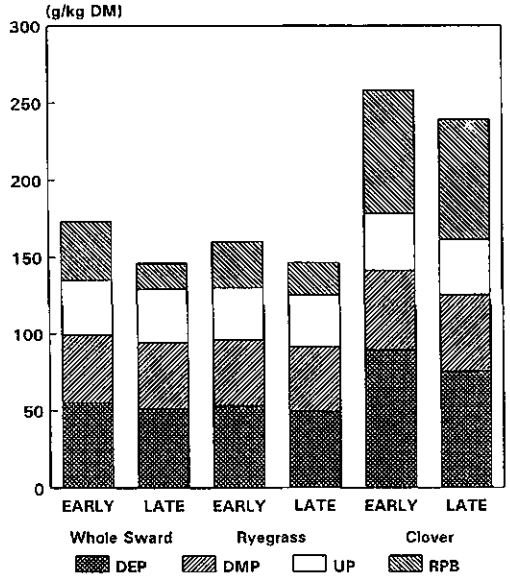
Intestinal protein digestion

The slightly higher fractions of feed protein escaping rumen degradation in grass compared to clover agree with findings of Beever *et al.* (1986) and Van Eys and Reid (1987). The average data on intestinal digestion of rumen escape protein of both grass and clover were lower than reported by Peyraud *et al.* (1988) but higher than values given by Varvikko and Vanhatalo (1989). Voigt *et al.* (1985) observed similar values. Differences may be due to feedstuff and experimental factors (Van Straalen *et al.*, 1993). Results of the mobile bag experiment are discussed in more detail by Van Straalen *et al.* (1993).

The data gathered were used to calculate amounts of intestinal digestible feed protein, intestinal digestible microbial protein, indigestible protein (feed and microbial origin) and, as difference with the CP content of the sample, rumen protein balance (Figure 5). Intestinal digestible microbial protein was calculated from the effectively fermentable OM, assuming an efficiency of 0.15, amino acid content of microbial CP of 0.75 and digestibility coefficient in the small intestine of 0.85, according to the Dutch protein evaluation system (Tamminga *et al.*, 1994). Indigestible protein was calculated as the sum of indigestibe feed and microbial protein.

The amount of intestinal digestible feed protein was consistently higher for clover than for grass by on average 30 g kg^{-1} DM. Maturition resulted in a decrease for clover but not for grass. The latter is in agreement with the results of Van Vuuren *et al.* (1991). Calculated intestinal digestible microbial protein from grass decreased as the season progressed by about 10 g kg^{-1} DM, as a consequene of an overall decrease in digestibility, whereas variation was very limited for clover. The predicted amounts of intestinal digestible protein varied from 89 to 104 and from 122 to 149 g kg^{-1} DM in grass and clover respectively. The rumen protein balance was clearly higher for clover than for grass, which agrees with higher NH_3 concentrations in clover diets compared with grass diets (Beever *et al.*, 1986). Therefore, to diminish undesired losses of N and increase microbial protein synthesis in the rumen, supplementation of clover-based rations with rumen-degradable carbohydrates seems advisable. For adequate rationing account should be taken of the proportion of clover in the sward. In the current experiment the level of intestinal digestible protein was calculated to be higher for mixed sward of Plot 2 than for Plot 1 by on average 6 g kg^{-1} DM. Simultaneously, average rumen protein balance increased by 9 g kg^{-1} DM.

Figure 5. Cumulative contents of small intestinal digestible escaped protein (SDEP), small intestinal digestible microbial protein (SDMP), small intestine non-digestible protein (SDNP), and rumen protein balance (RPB) for whole sward, ryegrass, and clover at two maturity levels



Conclusions

Limited differences were observed in chemical composition of grass swards intensively fertilized with N and of moderately N-fertilized ryegrass from mixed grass/clover swards. As a result, degradation characteristics were very similar throughout the season, with slight trends towards less variation in ryegrass. The DM of clover contained more CP and less cell walls, the latter clearly differing in composition from grass. The rumen-degradable fraction of both OM and CP was higher in clover than in grass, particularly due to larger instantly rumen-degradable fractions. The slowly degradable fraction of OM (not CP), in clover was also degraded faster, than in grass. The degradability of both forages declined during the season and as a consequence of later harvesting. Overall, effects were more limited for the grass/clover sward than for the grass sward, suggesting that animal production systems may profit from a less variable forage quality. As a consequence of higher protein content and differences in degradation, both rumen-degradable protein and rumen escape protein contents were higher in clover than in grass. Because, moreover, intestinal digestibility of escaped protein was higher, the amount of intestinal digestible feed protein was considerably higher in clover than in grass. As clover contained a higher level of rumen-fermentable carbohydrates, the inclusion of clover in swards may result in an increase in level of microbial protein as well. However, to avoid an increase in undesired losses of N in the

rumen, supplementation of clover-based rations with rumen-degradable carbohydrates seems advisable.

Acknowledgements

The authors wish to thank S. Tamminga (Department of Animal Nutrition, WAU) and A.M. van Vuuren (ID-DLO) for their contribution to the discussion and critical reading of the manuscript, and J. Kogut of the Agricultural Mathematics Group (GLW-DLO) for his statistical assistance. Furthermore the authors are grateful to R. Terluin (ID-DLO) and his team for taking care of the animals and assistance during the experiments. One of the authors (WvS) was partially sponsored by the Fund for Manure and Ammonia Research (FOMA).

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CHAPTER 5

INTESTINAL DIGESTIBILITY IN DAIRY COWS OF PROTEIN FROM GRASS AND CLOVER MEASURED WITH MOBILE NYLON BAG AND OTHER METHODS

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Published in:
Journal of Dairy Science (1993) 76, 2970-2981

INTESTINAL DIGESTIBILITY OF PROTEIN FROM GRASS AND CLOVER IN DAIRY COWS MEASURED WITH MOBILE NYLON BAG AND OTHER METHODS

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Abstract

Ruminal, intestinal, and total tract digestibilities of CP in grass, grass silage, and clover were measured using the mobile nylon bag method. Total tract indigestibilities measured with this method were compared with indigestible CP predicted by in vitro, 14-d ruminal incubation, and 12-h followed by 14-d ruminal incubation methods. Indigestible CP was related to chemical composition and harvest date.

Ruminal disappearance of CP from nylon bags was 58, 71, and 58% for grass, grass silage, and clover respectively. Intestinal CP disappearance was 84, 81, and 91% of escape CP, respectively. Lower ruminal disappearance was compensated by higher intestinal disappearance, resulting in a small variation in total tract indigestible CP within forage. Total tract indigestible CP was 7, 6, and 4%, respectively.

Average total tract indigestible CP measured with the mobile nylon bag method (6%) was lower than estimated by the in vitro (13%), 14-d ruminal incubation (14%) or 12-h ruminal incubation followed by 14-d ruminal incubation (11%) methods. All methods ranked forages as similarly, resulting in high correlation coefficients among methods within forage.

Total tract indigestible CP of grass, grass silage, and clover was related to CP content and harvest date, DM and ADIN content, and DM and CP contents, respectively.

Key words: protein, digestibility, forages, mobile nylon bag

Abbreviation key: E_{CP} = effective rumen escape CP, I_{CP} = indigestible CP (total tract), IV = *in vitro*, MNB = mobile nylon bag, PRI = prolonged ruminal incubation, SDE_{CP} = small intestine digestible escape CP, TT = transit time, 2SRI = two-step ruminal incubation.

Introduction

Feed protein digestion in ruminants consists of microbial fermentation in the rumen followed by enzymatic degradation and subsequent absorption in the small intestine. The extent of ruminal and intestinal degradation is dependent on both feedstuff and animal

characteristics (Van Straalen and Tamminga, 1990). However, total tract digestibility within a feedstuff is relatively constant (De Boer *et al.*, 1987, Hvelplund *et al.*, 1992). Recently developed protein evaluation systems for ruminants require data on the degradability of feed protein in the rumen and intestine, which can be estimated by *in vivo*, *in situ*, and *in vitro* (**IV**) methods.

In vivo methods are laborious, require cannulated cows, and can measure only the apparent digestibility of a limited number of feedstuffs. Widely accepted alternatives are *in situ* methods. With these methods, the CP disappearance of a large number of feedstuffs can be measured in the rumen (Ørskov and McDonald, 1979) and intestine using the mobile nylon bag method (**MNB**) (De Boer *et al.*, 1987, Hvelplund *et al.*, 1992), which is the standard in the new protein evaluation system in The Netherlands (Centraal Veevoeder Bureau, 1991). For forages, few data on small intestine digestibility of ruminal escape CP (SDE_{CP}) were available. Total tract indigestible CP (I_{CP}) therefore was predicted from prolonged ruminal incubation of 14-d (**PRI**), assuming that this residue was related closely to I_{CP}, measured with the MNB method, as for concentrate feedstuffs (Tamminga and Ketelaar, 1988). However, this relationship was not investigated for forages.

Recently, IV techniques using proteolytic enzymes have been developed to estimate protein degradability in the rumen (Aufrère *et al.*, 1990) and intestine (Antoniewicz *et al.*, 1992). Such laboratory procedures are relatively easy to standardize and do not require animals, as do *in vivo* or *in situ* methods. For concentrate feedstuffs, the IV method to estimate intestinal digestibility showed a close relationship with the MNB method (Antoniewicz *et al.*, 1992), but this relationship has not been established for forages.

Tamminga *et al.* (1991) showed that the undegradable CP fraction of forages in the rumen was related to DM, CP, and NDF contents, and to harvest date. The I_{CP} measured with the MNB method could be related to the ADIN content of the feedstuff, although with variable success (Aufrère *et al.*, 1991, Peyraud *et al.*, 1988b).

The objective of this study were 1) to evaluate the MNB method to measure SDE_{CP} and I_{CP} of grass silage, grass, and clover; 2) to compare the results of the MNB method with those of other methods; and 3) to investigate the relationship between I_{CP} measured with the MNB method and the chemical composition of the forages.

Materials and methods

Samples

Samples of grass silage were obtained from different experiments carried out at the Research Institute for Livestock Feeding and Nutrition Research and the Wageningen Agricultural University (Bosch *et al.*, 1991). In these experiments, the degradation of CP

in the rumen was measured using the rumen nylon bag technique (Van Vuuren *et al.*, 1989).

Samples of grass and clover were obtained from an experiment carried out by Steg *et al.* (1994). In that experiment, seven samples of herbage from two plots were collected from June 5 to September 11, 1990. One plot was sown with *Lolium perenne L.* cv. Profit and Magella and fertilized with 315 kg N/ha per yr (whole sward) and the other with a mixture of *Lolium perenne* and *Trifolium repens L.* cv. Retor, fertilized with 91 kg N/ha per yr. Swards were sampled at a light (1700 to 2000 kg of DM/ha) and heavy yield (>3000 kg of DM/ha). Herbage samples of the second plot were separated into ryegrass and clover. The proportion of clover ranged from .141 to .294 g/g of DM herbage. Detailed information about the experimental procedures and ruminal degradation of CP of grass and clover samples was given by Steg *et al.* (1994).

Samples were cut with a paper guillotine to a particle length of approximately 1 cm and stored at -18°C. Chemical composition and harvest date of the samples from grass silage, whole sward, ryegrass, and clover are given in Table 1.

MNB Method

Two experiments were carried out to measure the intestinal digestibility with the MNB method. In Experiment 1, the grass silage samples were used. Residues of ruminal incubation were obtained, using the rumen nylon bag technique described by Van Vuuren *et al.* (1989). Rumen nylon bags (9 x 18 cm, polyamide, porosity 30%, mesh size 40 μm; Nybolt, Zurich, Switzerland), were filled with fresh sample (equivalent to 5 g of DM) and incubated in the rumen of four dairy cows for 6, 9, 12, 15, and 18 h. After incubation, bags were washed for 50 min with 60 L of cold water without spinning in a domestic washing machine (Van Vuuren *et al.*, 1989) and subsequently freeze-dried. Residues were pooled per sample and per cow and ground over a 3-mm sieve. Part of the residue was further ground (1 mm) and used for DM, ash, and N analyses.

MNB (3 x 6 cm, same material as rumen nylon bags) were filled with .5 g of the freeze-dried and ground ruminal residue. Bags were closed by heat sealing. Sixteen bags per sample and per cow were used, four bags for each ruminal residue of each cow, for a a total of 448 bags. Prior to intestinal incubation, bags were incubated in an HCl solution (.1 *M*) with pepsin [1 g/L, 2000 FIP-U/g (units according to Fédération Internationale Pharmaceutique), Merck, Darmstadt, Germany] for 1 h at 37°C. Bags were inserted via a T-cannula in the distal duodenum during two periods (600 to 1200 h and 1400 to 2000 h) on 2 d (d 1 and 3). Every 20 min, 4 bags per cow were introduced. Replicate bags were divided over the four incubation periods. Feces were collected every 2 h, from 6 h after the first bag had been introduced into the duodenum until 48 h after the insertion of the last bag. Bags were recovered from the feces as feces were washed over a 5-mm screen; bags were cleaned with water and stored at -18°C. When the collection of the faeces was stopped, bags were thawed and washed in a domestic washing machine using 120 L of

Table 1. Concentration of DM, OM, CP, NDF, and ADIN and harvest date of grass silage, whole sward, ryegrass, and clover

Sample	No.	DM (%)	OM	CP	NDF	ADIN (% N)	Harvest date
			--------- (% of DM) -------				
Grass silage	1	46.0	90.4	18.6	36.7	2.4	May 8
	2	47.1	90.3	15.8	42.3	2.2	May 16
	3	54.3	90.8	19.6	54.7	2.9	May 28
	4	33.7	-	23.2	41.6	3.6	Jul 1
	5	59.4	86.8	213	44.6	1.8	Jul 2
	6	55.0	92.6	11.2	67.3	5.3	Jul 6
	7	60.8	89.8	20.9	54.8	3.0	Aug 8
Mean		50.9	90.1	18.7	48.9	3.0	
Whole sward	1	15.1	88.0	17.3	42.7	1.7	Jun 5
	2	14.6	89.4	13.6	49.4	2.6	Jun 20
	3	16.7	88.5	18.2	48.4	3.2	Jun 26
	4	16.2	89.5	14.5	54.9	5.0	Jul 10
	5	23.3	88.7	16.7	53.1	4.3	Jul 17
	6	24.0	89.6	14.4	52.1	5.1	Jul 31
	7	16.0	88.8	16.8	56.8	5.8	Sep 4
Mean		18.0	88.9	15.9	51.1	4.0	
Ryegrass	1	17.4	87.5	16.8	44.4	1.9	Jun 5
	2	14.7	88.1	13.5	49.4	4.5	Jun 20
	3	18.2	88.2	14.1	49.4	4.0	Jun 26
	4	16.3	88.3	14.3	54.0	5.8	Jul 10
	5	24.6	87.6	15.1	52.0	4.8	Jul 17
	6	23.7	88.0	14.3	52.1	6.0	Jul 31
	7	15.0	87.4	17.8	57.8	5.4	Sep 11
Mean		18.6	87.9	15.1	51.3	4.6	
Clover	1	13.4	87.3	27.5	22.0	2.1	Jun 5
	2	11.2	87.1	24.4	26.3	4.4	Jun 20
	3	12.1	87.1	26.4	26.0	4.3	Jun 26
	4	11.7	87.8	24.0	34.5	5.5	Jul 10
	5	17.7	88.0	25.9	33.9	5.1	Jul 17
	6	18.3	89.0	22.8	37.0	5.6	Jul 31
	7	15.2	88.7	23.2	35.4	4.7	Sep 11
Mean		14.2	87.9	24.9	30.7	4.5	

water at 15°C without spinning. After washing, bags were dried at 70°C, and residues were pooled per sample and per cow, ground over a 1-mm sieve, and analyzed for DM, ash, and N.

In Experiment 2, samples of whole sward, ryegrass and clover were incubated. The procedure differed from Experiment 1: ruminal incubation was carried out during 12 h with five replicates for grass and seven for clover samples. One incubation period instead of five was used because of the larger number of samples in experiment 2. The length of incubation (t = 12 h) was based on the average degradation curve of grasses (Van Straalen and Tamminga, 1990) and calculated from

$$\text{residue}_t = U_{CP} + D_{CP} \times e^{-(kd \times t)}$$

where

U_{CP} = undegradable fraction,
D_{CP} = potentially degradable fraction, and
k_d = degradation rate of D_{CP},

which results in

$$t = (\ln(\text{residue}_t - U_{CP}) - \ln(D_{CP}))/-k_d.$$

The incubation time was calculated assuming that residue_t was equal to the effective escape of CP (E_{CP}) from the rumen, calculated according to Ørskov and McDonald (1979), with a passage rate of 4.5%/h (Van Straalen and Tamminga, 1990). The ruminal CP residues were compared with the calculated E_{CP} to validate whether these residues were representative for E_{CP}.

For intestinal incubation, 6 bags per cow and sample (total of 504 bags) were used, and the ruminal residue of each cow was incubated in the intestine of each cow. Because all washable material may not have been removed by the washing procedure in Experiment 1, a more extensive program was used in the Experiment 2: 100 L of water at 40°C with spinning (700 rpm).

Other Methods

The I_{CP} measured with the MNB technique was compared with I_{CP} determined by an IV method, PRI method, and two stage ruminal incubation (**2SRI**) method (12-h ruminal incubation followed by freezed drying, grinding, and 14-d ruminal incubation).

For the IV method, ruminal residues, prepared as described in the rumen nylon bag method, were pooled per sample. The IV degradation was measured according to the method of Antoniewicz *et al.* (1992) using three to five replicates. The N solubilization

after pepsin-HCl incubation followed by pancreatin incubation is measured.

The undegradable CP fraction after PRI of the original samples was obtained from previous experiments (Bosch *et al.*, 1991, Steg *et al.*, 1994, A.M. van Vuuren, 1988, unpublished data; H. de Visser, 1988, unpublished data). To enable a better comparison between MNB, IV and PRI methods, the residues after ruminal incubation (12 h) used in the MNB experiment were also incubated in the rumen for 14 d (2SRI method). Freeze-dried and ground ruminal residues (5 g) were weighed into nylon bags (one replicate per sample) and incubated in the rumen of 1 cow for 14 d. Washing procedure was similar to that for ruminal residues of MNB method.

Cows

In each of the two MNB experiments, 4 dairy cows were surgically prepared with large rumen cannulas (Bar Diamond, Parma, ID) and T-type cannulas (made at our institute) in the proximal duodenum. In Experiment 1, cows were fed grass silage and concentrate; in Experiment 2, they received dried grass and concentrates (forage to concentrate ratio of 40:60). In each experiment, one cow was lactating and received approximately 15 kg/d of DM and the others were nonlactating, consuming approximately 10 kg/d of DM.

Chemical Analysis

Forages and incubation residues were analyzed for DM, ash, and Kjeldahl N according to the standard procedures at our institute (Steg *et al.*, 1990). Forages were analysed further for NDF (Robertson and Van Soest, 1981) and ADIN (Goering and Van Soest, 1970).

Statistical Analysis

Analysis of variance was used to determine the effects of forage (grass silage, whole sward, ryegrass, and clover) and cow on the results of the MNB experiment according to the following model:

$$Y_{ijk} = \mu + R_i + C_j + RC_{ij} + e_{ijk}$$

where

Y_{ijk} = disappearance of DM and CP after ruminal, intestinal, or total tract incubation,
μ = mean,
R_i = roughage effect (i = 1...4),
C_j = cow effect (j = 1...4),
RC_{ij} = interaction between roughage and cow, and

e_{ijk} = residual error, assumed to be normally and independently distributed.

Regression analysis was used to obtain the relationship among 1) residual CP after ruminal incubation and the calculated E_{CP}; 2) transit time (TT) of MNB in the intestine and DM disappearance from these bags; and 3) residual CP after ruminal and intestinal incubation.

The difference in I_{CP} between methods (MNB, IV, PRI, and 2SRI) and forages (grass silage, whole sward, ryegrass, and clover) were tested using ANOVA:

$$Y_{ijk} = \mu + R_i + M_j + RM_{ij} + e_{ijk}$$

where

Y_{ijk} = I_{CP},
μ = mean,
R_i = roughage effect (i = 1...4),
M_j = method effect (j = 1...4),
RM_{ij} = interaction between roughage and method, and
e_{ijk} = residual error, assumed to be normally and independently distributed.

Correlation coefficients were calculated among I_{CP} measured by different methods for data sets, included all forages and separated for each forage. Using multiple regression, equations were derived to predict I_{CP} measured with the MNB technique by chemical composition (DM, CP, NDF, and ADIN contents) and harvest date for each roughage. Statistical calculations were carried out using Genstat software (Genstat 5 Committee, 1987). Significance was declared at $P < .05$, unless stated otherwise.

Results and discussion

MNB Method

The average disappearance of DM and CP from nylon bags in the rumen, intestine, and total tract is given in Table 2. The average and standard deviation of ruminal and intestinal disappearance of DM and CP per sample is shown in Figures 1, a and b, respectively. Ruminal disappearance of DM after 12 h of incubation of both grasses was lower ($P < .001$) than of grass silage (average of 6, 9, 12, 15, and 18 h incubations). Grass silage showed a lower ruminal disappearance of DM than did clover ($P < .001$). Ruminal CP disappearance of both grasses and clover was not different, but grass silage showed higher ($P < .001$) CP disappearance.

Table 2. Average disappearance of DM and CP in the rumen (% of original forage), intestine (% of rumen residual) and total tract (% of original forage) of grass silage, whole sward, ryegrass and clover measured with the mobile nylon bag method

Site	Component	Grass silage	Whole sward	Ryegrass	Clover	SED[1]
		---------- (%) ----------				
Rumen[2]	DM	48.2[b]	38.5[c]	38.8[c]	54.5[a]	2.1
	CP	70.6[a]	57.5[b]	57.4[b]	57.8[a]	2.1
Intestine	DM	28.0[c]	40.8[b]	41.9[b]	68.9[a]	1.4
	CP	81.4[c]	83.4[b]	84.3[b]	91.4[a]	1.0
Total tract	DM	62.4[b]	63.4[b]	64.3[b]	85.7[a]	.8
	CP	94.6[c]	92.8[b]	93.3[b]	96.3[a]	.5

[a, b, c] Figures with different superscript in the same row differ significantly ($P < .05$).

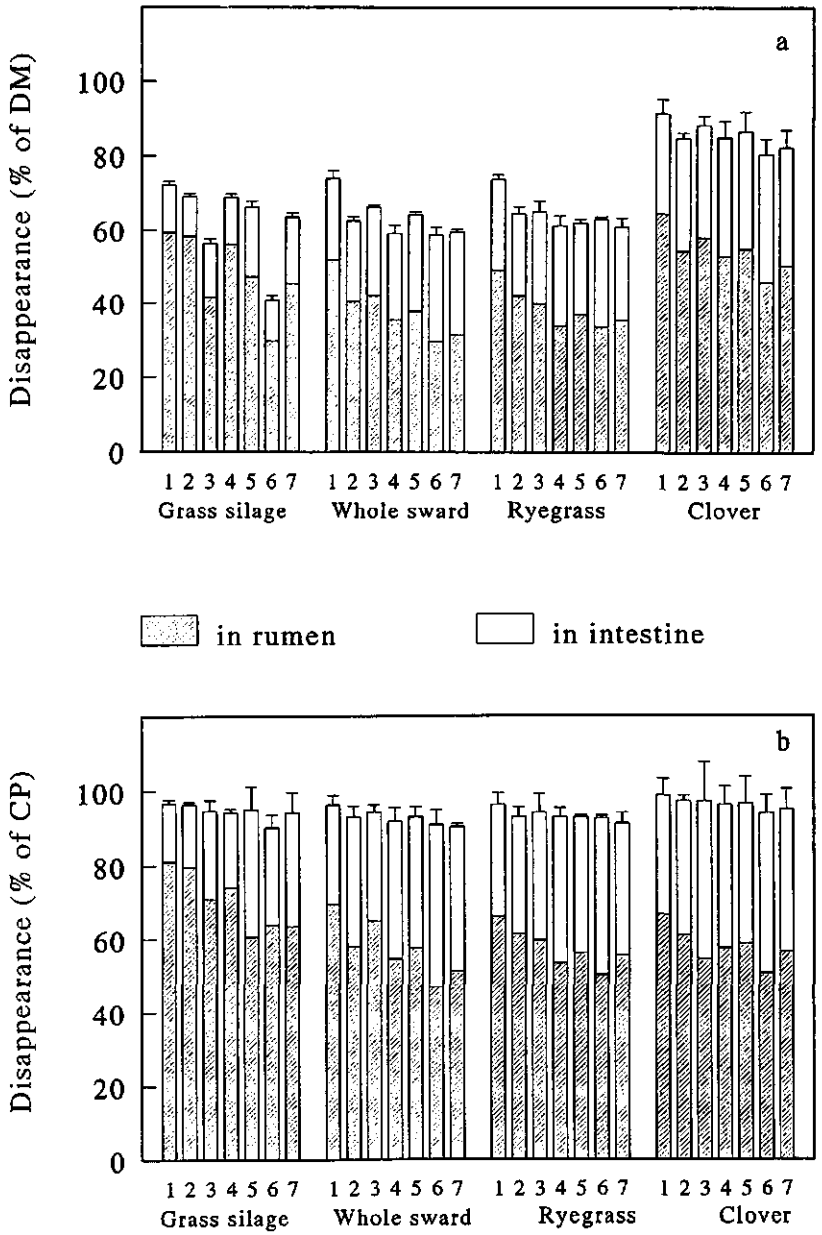
[1] Standard error of difference.

[2] For grass silage: average of 6, 9, 12, and 15 h of incubation; for whole sward, ryegrass, and clover: after 12 h incubation.

Residual CP after ruminal incubation in the MNB method was higher than the E_{CP} calculated from the degradation characteristics of the same samples and an assumed passage rate of 4.5%/h (20). The average difference was larger for grass silage and clover (4.5%) than for both grasses (2.5%). The relationship between residual CP and E_{CP} as a percentage of CP using all data ($n = 28$) was residual CP = 5.7 ($\pm$ 3.8) + .934 ($\pm$ .104) x E_{CP} ($R^2 = .75$, CV = 16%). This implies that ruminal incubation time in our experiments was too short to obtain CP residues comparable to E_{CP}.

The number of MNB incubated per day per cow in our experiments was much larger than in other experiments (Hvelplund *et al.*, 1992, Voigt *et al.*, 1985). Impaired intestinal function was not indicated in our experiments, or in experiments in which MNB were incubated at the same frequency (Tamminga and Ketelaar, 1988). The recovery of MNB in the feces was 99% in Experiment 1 and 97% in Experiment 2. Of the recovered bags, 9 and 6%, respectively, were ruptured after washing. The mean TT ($\pm$ SD) was 13.7 $\pm$ 4.2 h in Experiment 1 and 18.8 $\pm$ 6.5 h in Experiment 2. The TT was in the range of means reported (Hvelplund, 1985, Voigt *et al.*, 1985). Intake of the cows can affect TT (Tamminga and Ketelaar, 1988), but, in our experiments, no difference was observed between the lactating and nonlactating cows. No relationship existed between TT and disappearance of DM from MNB, which is in contrast with results of Hvelplund (1985). Voigt *et al.* (1985) found a relationship of TT with DM disappearance, but not with CP

Figure 1. Disappearance of DM (a) and CP (b) in the rumen and intestine measured by the mobile nylon bag method of grass silage, whole sward, ryegrass, and clover (numbers refer to sample number in Table 1; vertical error bars represent standard error)



disappearance. The pooling of intestinal residues per sample and cow before N analysis in our experiment, without consideration of TT, was, therefore, regarded as valid.

To reduce microbial contamination and to remove degraded feed and endogenous material, both rumen bags and MNB were washed intensively. Microbial contamination was not measured in our experiments, but, for forages incubated for 12 h in the rumen, Nocek and Grant (1987) observed microbial contaminations of 0 to 60% of the residual N. For residues of MNB, reported microbial contamination was limited (Hvelplund, 1985, Kohn and Allen, 1992). The washing procedure after intestinal incubation in our experiments was more intensive than after ruminal incubation because all soluble material may not have been removed from MNB by the ruminal washing procedure (W.M. van Straalen, 1990, unpublished data). This problem was not observed using concentrate feedstuffs (S. Tamminga, 1990, personal communication). The intensive washing procedure could have resulted in loss of small particles. However, for concentrate feedstuffs, the disappearance of CP from MNB using the intensive washing procedure (W.M. van Straalen, 1991, unpublished data) was similar to what was observed previously with the less intensive washing procedure (Tamminga and Ketelaar, 1988). The higher fiber content and larger volume of forage intestinal residues compared to concentrate residues may have caused the different effects of the washing procedure.

Intestinal disappearance of rumen residual DM and CP was higher for clover ($P < .001$) than for ryegrass and whole sward, which were higher ($P < .001$) than for grass silage (Table 2). The coefficient of variation between replicates of one sample for SDE_{CP} (4 cows) varied from .1 to 4.1% (average 1.5%) (Figure 1b).

The average SDE_{CP} of grass silage was higher than observed by Varvikko and Vanhatalo (Varvikko and Vanhatalo, 1991). For grasses and white clover, SDE_{CP} values were higher, similar, and lower (Peyraud *et al.*, 1988a; Varvikko and Vanhatalo, 1989; Voigt *et al.*, 1985). Differences between experiments can be caused by pretreatment of samples (and ruminal residues), mesh size of the bags, sample quantity, and ruminal incubation time (De Boer *et al.*, 1987; Hvelplund *et al.*, 1992). In Experiment 1, two silages also were freeze-dried and ground before ruminal incubation, which resulted in higher ruminal, but lower intestinal, CP disappearance than for fresh and chopped equivalents.

As a result of ruminal and intestinal disappearance, total tract DM disappearance from bags was higher for clover ($P < .001$) than for whole sward, ryegrass, and grass silage. Total tract CP disappearance was higher for clover than for grass silage ($P < .001$), followed by both grasses ($P < .01$), and variation within each forage was small. The correlation coefficients between CP disappearance in the rumen and in the intestine (expressed as fraction of escape CP) were -.06, .80, .40, and .77 for grass silage, whole sward, ryegrass, and clover, respectively. When the disappearance in the intestine was expressed as fraction of original CP ($1 - I_{CP}$), these relationships were -.97, -.99, -.97, and -.98, respectively. The high correlation coefficients indicate that, within each forage, lower ruminal disappearance was compensated by higher intestinal disappearance, which

resulted in a relatively constant I_{CP}. This compensation also occurs when ruminal CP disappearance was varied by incubation of feedstuffs for different times in the rumen (De Boer *et al.*, 1987, Hvelplund *et al.*, 1992). This finding supports the hypothesis of Hvelplund *et al.* (1992) that each feedstuff contains a CP fraction that is undegradable in both the rumen and intestine, and also suggests that the intestine has a more than sufficient capacity to digest protein. For most feedstuffs, only a small difference in I_{CP} was observed between preincubated samples and samples that were not incubated in the rumen (De Boer *et al.*, 1987, Hvelplund *et al.*, 1992). This difference increased when ruminal incubation time increased and was larger for forages than for concentrates. When I_{CP} was measured with the MNB method, ruminal incubation time is of only minor importance and probably can be omitted.

The storage of forages by freezing resulted in a lower buffer solubility of CP and higher neutral detergent insoluble CP compared to fresh samples, probably caused by precipitation of protein, but did not affect the ADIN content (Kohn and Allen, 1992). However, the effect of this preservation method, on ruminal and intestinal digestibility of CP has not been investigated and remains unclear.

In most protein evaluation systems, both E_{CP} and SDE_{CP} are considered to be constant per feedstuff, regardless of feed quality or feeding conditions. Exceptions are the new Dutch protein evaluation system (Centraal Veevoeder Bureau, 1991), in which E_{CP} for forages is variable and estimated from the chemical composition and harvest date (Van Straalen and Tamminga, 1990), and the Agricultural Research Council system (1984), which uses variable E_{CP} for the same feedstuff through the application of different passage rates, which are dependent on production level. In those cases, the SDE_{CP} also should be variable within a feedstuff and can be estimated from the actual E_{CP} and constant I_{CP}: $(E_{CP} - I_{CP})/E_{CP}$.

Other Methods

The I_{CP} measured with the MNB method was lower ($P < .001$) than with the IV method (Table 3). Differences among methods can be caused by differences in pepsin-HCl incubation and enzyme activities and by hindgut fermentation, resulting in microbial and endogenous contamination of MNB residuals. The pepsin-HCl incubation simulates abomasal digestion, but the effect of this incubation on intestinal digestibility is not clear. Without pepsin-HCl incubation, Voigt *et al.* (1985) observed intestinal digestibilities similar to those in our experiment. The concentration of pepsin in the MNB method was based on previous experiments and was higher than used in the IV method, but the incubation was shorter (1 h in MNB and 2 h in IV method). Hindgut fermentation has only a limited effect on intestinal disappearance of CP (Hvelplund, 1985, Voigt *et al.*, 1985). Considerable microbial and endogenous contamination, if present, would have resulted in a higher I_{CP} measured with the MNB than with the IV method, which was in contrast to that observed. Both types of contaminations are influenced by the washing

procedure. Among methods, I_{CP} showed a significant correlation for data sets of grass silage, whole sward, and clover, but not for ryegrass (Table 4; Figure 2a). With concentrate feedstuffs, agreement between MNB and IV methods was close; regression coefficient was close to 1 (Antoniewicz *et al.*, 1992).

Table 3. Average and statistical differences of total tract indigestible CP measured by the mobile nylon bag (MNB), *in vitro* (IV), prolonged ruminal incubation (PRI), and 2 stage ruminal incubation (2SRI) method of grass silage, whole sward, ryegrass and clover[1]

	Method				
Forage	MNB	IV	PRI	2SRI	Mean
	(% of CP)				
Grass silage	5.5	10.9	10.6	11.0	9.5[x]
Whole sward	7.2	15.1	18.1	12.9	13.3[y]
Ryegrass	6.8	13.8	17.4	12.2	12.6[y]
Clover	3.7	10.0	10.0	9.4	8.3[x]
Mean	5.8[a]	12.5[cd]	14.0[d]	11.4[c]	10.9

[x, y] Forage-effect: figures with different superscripts in column are different ($P < .05$).
[a, b, c] Method-effect: figures with different superscripts in row are different ($P < .05$).
[1] Standard error of difference (SED): among forages SED = 1.0; among methods SED = 1.0; among forages and methods: = 2.0

Measured with the MNB method, I_{CP} was lower ($P < .001$) than with the PRI and 2SRI methods (Table 3). This difference can be caused by difference in washing procedure or by the grinding of ruminal residues (only MNB and PRI method). Although grinding of ruminal residues in the MNB method was included because of methodological aspects, it can considered to be a simulation of the rumination process. The lower I_{CP} measured with the MNB method compared with the 2SRI method indicates that the residue of PRI is still partly digestible in the intestine. The correlation between I_{CP} measured by the MNB and PRI or 2SRI methods was high and significant, except for the MNB and 2SRI method with the grass silage data (Table 4; Figure 2, b and c), which agreed with results obtained by Peyraud *et al.* (1988b). The close relationship between the MNB and PRI method supports the assumption in the Dutch protein evaluation system that SDE_{CP} of forages can be estimated from E_{CP} and the residual CP after 14 d ruminal incubation (Centraal Veevoeder Bureau, 1991). Because of the low regression coefficient (.33) (Figure 2b), an equation should be used when SDE_{CP} is estimated in this way.

Figure 2. Relationships between total tract indigestible CP (I_{CP}) measured by the mobile nylon bag (MNB) method and *in vitro* (IV) method (MNB = -1.6 + .589 x IV; R^2 = .82) (a), prolonged ruminal incubation (PRI) method (MNB = 1.2 + .327 x PRI; R^2 = .76) (b), and 2 stage ruminal incubation (2SRI) method (MNB = 1.2 + .408 x 2SRI; R^2 = .64) (c) of grass silage (+); whole sward (△); ryegrass (○); clover (□)

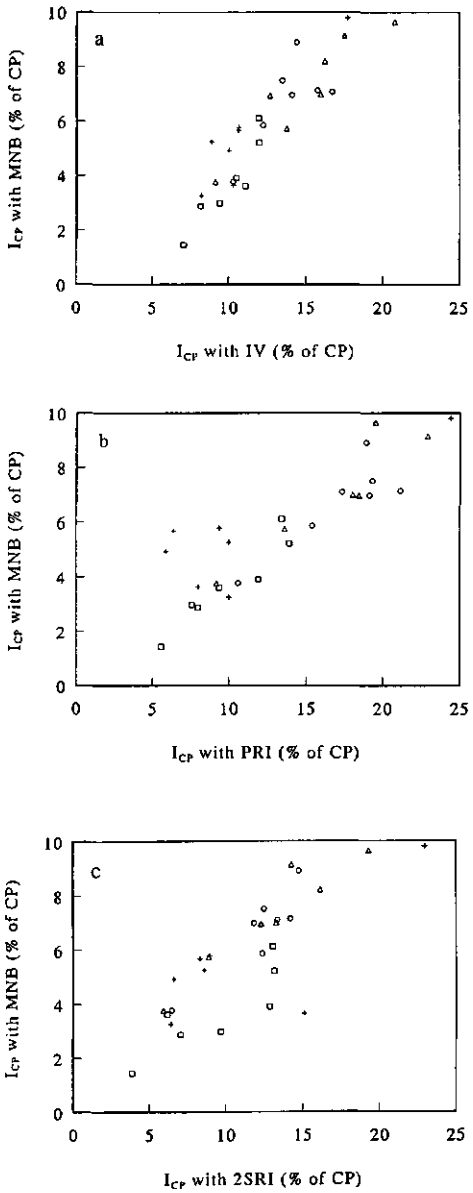


Table 4. Correlation coefficients between total tract indigestible CP measured by the mobile nylon bag (MNB), *in vitro* (IV), prolonged ruminal incubation (PRI), and 2 stage ruminal incubation (2SRI) method for all forages, grass silage, whole sward, ryegrass, and clover

Forage	Method	MNB	IV	PRI
All forages	IV	.91***		
	PRI	.88***	.87***	
	2SRI	.81***	.82***	.79***
Grass silage	IV	.93**		
	PRI	.83*	.88**	
	2SRI	.72NS	.90**	.85*
Whole sward	IV	.94**		
	PRI	.86*	.71NS	
	2SRI	.95***	.93**	.82*
Ryegrass	IV	.71NS		
	PRI	.85*	.78*	
	2SRI	.91**	.80*	.87*
Clover	IV	.92**		
	PRI	.95**	.91**	
	2SRI	.85*	.77*	.89**

NS $P > .05$; * $P < .05$; ** $P < .01$; *** $P < .001$.

The mean I_{CP} measured with the PRI method was higher than with the 2SRI method (Table 3). Thus, treatment of ruminal residues (freeze-drying and grinding) resulted in further loss of CP in the rumen. Robinson *et al.* (1986) reported a loss of 24% of the neutral detergent residue from undegradable residue (42 d) from ryegrass when residues were ground (1 mm) and reincubated in the rumen. The extra CP disappearance from nylon bags after drying and grinding also can be due to extra loss of undegradable particles < 41 μm. The treatment also may have affected the availability of CP for enzymatic degradation by changing protein structure or by liberating protein through damage of cell walls.

The I_{CP} measured with the IV method was not different from the PRI and 2SRI methods and showed a close relationship, except for IV and PRI methods for the whole sward data set (Tables 3 and 4).

Prediction of I_{CP}

The I_{CP} of forages in our experiments showed a close relationship with chemical composition and harvest date. Simple correlations between I_{CP} and NDF were .86 for all forages, .88 for grass silage, .91 for whole sward, .94 for ryegrass, and .91 for clover. Between I_{CP} and ADIN, correlations were .59, .91, .92, .89, and .80 respectively. In Figure 3, the relationship between ADIN x 6.25 and I_{CP} is presented. For whole sward and ryegrass, I_{CP} was positively related to harvest date; for clover I_{CP} was positively related to harvest date and negatively to CP content. Correlation coefficients between ADIN and NDF were .26, .75, .94, .84, and .87, respectively, for all forages, grass silage, whole sward, ryegrass, and clover. Goering and Van Soest (1970) proposed the ADIN as measure for the I_{CP} after heat treatment of forages. The method was used to estimate SDE_{CP} and I_{CP} of a large range of feedstuffs with variable success (Aufrère *et al.*, 1990, Peyraud *et al.*, 1988b). The mean ADIN x 6.25 in our experiments was lower than I_{CP} for grass silage, whole sward, and ryegrass but higher for clover. In agreement with results of Peyraud *et al.* (1988b), our results indicate that ADIN can only be used to estimate the I_{CP} within one feedstuff or a group of similar feedstuffs.

Figure 3. Relationship between total tract indigestible CP (I_{CP}) measured by the mobile nylon bag (MNB) method and ADIN x 6.25 content (MNB = 2.0 + .927 x ADIN x 6.25; R^2 = .32) of grass silage (+); whole sward (△); ryegrass (○); clover (□)

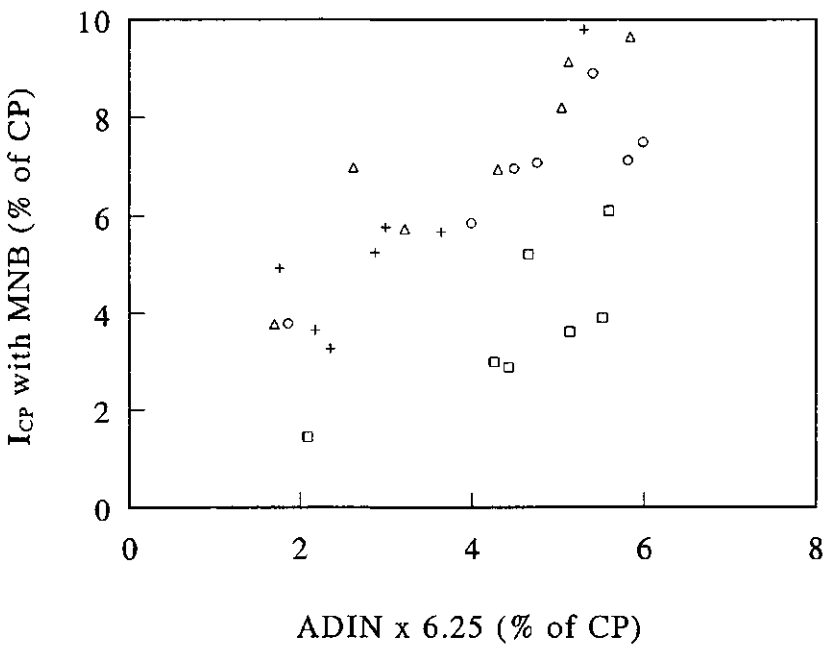


Table 5. Coefficients of multiple regression equations to predict total tract indigestible CP (% of CP) measured by the mobile nylon bag method from DM, CP, and ADIN contents and harvest date of grass silage, whole sward, ryegrass, and clover

Forage	a[1]	SE	DM b_1	SE	CP b_2	SE	ADIN b_3	SE	Harvest date b_4	SE	R^2	RSD[2]
Grass silage	-3.2	2.1	.072	.030			1.730	.242			.90	.7
Whole sward	9.6	2.0			-.530	.113			.584	.066	.94	.5
Ryegrass	8.4	2.1			-.478	.157			.535	.077	.88	.5
Clover	17.9	3.5	.211	.074	-.691	.122					.89	.5

[1] a = intercept; b_1 = regression coefficient for DM (%); b_2 = regression coefficient for CP (% of DM); b_3 = regression coefficient for ADIN (% of N); b_4 = regression coefficient for harvest date (days elapsed since the first of April).

[2] RSD = Residual standard deviation.

[3] SE = standard error of estimate.

The coefficients of the multiple regression equations to predict I_{CP} with the highest R^2 are given in Table 5. The I_{CP} of grass silage increased with increasing DM and ADIN contents. Tamminga *et al.* (1991) also observed a positive effect of DM content on undegradable CP after PRI of grass silages. For both grasses, I_{CP} decreased with increasing CP content and increased during the season. Van Straalen and Tamminga (1990) reported a similar relationship between undegradable CP after PRI and CP and harvest date. High CP contents in grass can be the result of harvesting at a young stage of maturity and high N fertilization level. During the season, NDF increased and CP decreased, resulting in more CP associated with cell walls and an increased ADIN to ADF ratio (Steg *et al.*, 1994). Cell-wall CP is digested slowly and has a larger undegradable fraction than CP that is not associated with cell walls. Results agree with Tamminga *et al.* (1991), who observed a positive relationship between undegradable CP after PRI and NDF content. In both cases, protein associated with cell walls is relatively smaller than at low CP content. For clover, I_{CP} increased as DM content increased and decreased as CP content increased, which also can be explained by the fraction of CP that is associated with cell walls.

Conclusions

Within a forage, lower ruminal disappearance of CP was compensated by higher intestinal disappearance of CP, resulting in a small variation in I_{CP}, thus validating the assumption that protein in a feedstuff contains a fraction that is undegradable in both the rumen and intestine (Hvelplund *et al.*, 1992). Therefore the SDE_{CP} can be calculated from $(E_{CP} - I_{CP})/E_{CP}$. The mean I_{CP} measured with the MNB method was lower than that measured with IV, PRI, or 2SRI methods. The high correlation coefficients among methods indicate that for routine analysis of feedstuffs, I_{CP} can be estimated by PRI or IV methods. The I_{CP} was higher from grass than for grass silage or clover and can be predicted by chemical composition and harvest date.

Acknowledgements

The authors thank S. Tamminga (Agricultural University Wageningen) for his contribution to the discussion and for critically reading the manuscript, and R. Terluin and his team for taking care of the cows and for assistance during the experiments. The assistance of G. Huisman during the first experiment and J. Kogut (Agricultural Mathematics Group) during statistical analysis is highly appreciated. This study was financed partly by Fonds Onderzoek Mest en Ammoniakproblematiek (Fund for Manure and Ammonia Research).

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CHAPTER 6

DIGESTION OF FEED AMINO ACIDS IN THE RUMEN AND SMALL INTESTINE OF DAIRY COWS MEASURED WITH NYLON BAG TECHNIQUES

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Submitted for publication

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Summary

The disappearance of total N, non protein-N and amino acid-N after washing, rumen incubation and intestinal passage of beetpulp, maizeglutenfeed, maize feed meal, palmkernel meal, soya bean hulls, soya bean meal, grass silage, maize silage and concentrate was measured in four dairy cows using nylon bag techniques. Disappearance of amino acid-N and non protein-N after washing varied between feedstuffs from 14 to 69 % and from -2 to 100% of feed amino acid-N and non protein-N, respectively. For beetpulp, grass silage and maize silage washing showed a considerable effect on the amino acid profile. Disappearance of amino acid-N and non protein-N after rumen incubation varied between feedstuffs from 25 to 71% and 18 to 89%, respectively. Rumen incubation had only a small effect on the amino acid profile of the residue after washing. Disappearance of amino acid-N in the intestine varied between feedstuffs from 72 to 99% of rumen undegraded amino acid-N, and was higher than the disappearance of non protein-N (38 to 97%). Intestinal incubation showed a considerable effect on the amino acid profile for all feedstuffs. It was concluded that protein that was assumed to escape rumen degradation and was absorbable in the intestine was higher in total amino acids and methionine, and lower in non protein-N and glutamic acid and proline compared to protein in the feedstuff.

Key words: amino acids digestion : nylon bags : feedstuffs : dairy cows

Introduction

Modern protein evaluation systems for ruminants describe the supply and requirement of true protein that is absorbable from the small intestine. To increase the efficiency by which this true protein is used for production purposes, research has been focused on the requirement of first limiting amino acids (Schwab *et al.*, 1992), and results are being incorporated into protein evaluation systems (Rulquin *et al.*, 1993). To meet requirements, the supply of absorbable individual amino acids can be altered by changing the source of undegraded protein in the diet (Seymour *et al.*, 1990). Before protein is absorbed in the small intestine, it is subjected to microbial fermentation in the rumen and enzymatic digestion in the intestine. However, the effect of rumen fermentation on the amino acid profile of undegraded protein is not clear. Some authors found no effect (Varvikko *et al.*, 1983; Weakley *et al.*, 1983; Messman, 1992), while others reported

different effects for different feedstuffs (Ganev *et al.*, 1979; Rooke, 1985; Crooker *et al.*, 1986; Hennessy *et al.*, 1983; Susmel *et al.*, 1989). Hardly any research has been carried out to study the effect of intestinal digestion on the amino acid profile of undegraded protein (Le Henaff *et al.*, 1988).

The effect of rumen fermentation and small intestine digestion on the amino acid profile of feed protein can be studied using the rumen nylon bag and mobile nylon bag technique (Van Straalen *et al.*, 1993). With these techniques dietary ingredients are generally incubated separately. Murphy and Kennelly (1987) and Stallings *et al.* (1991) showed that rumen degradation of protein in a concentrate or complete diet mixture could be predicted from the degradation of the ingredients of the concentrate or diet, but for intestinal digestion this was never tested.

The objective of the present study was to determine the effect of washing, rumen incubation and intestinal incubation on the amino acid profile of feedstuffs using nylon bag techniques, in order to predict the supply of individual amino acids to the animal from rumen undegraded feed. Also a comparison was made between measured residues after washing, rumen incubation and intestinal incubation of amino acids of a concentrate, and the estimated values from the ingredients of the concentrate.

Materials and methods

Experimental animals

Four dairy cows (HF) were used, of which three were lactating and one was non-lactating. All animals were fitted with a permanent rumen cannula (Bar Diamond Inc., Pharma, Idoha, USA) and a T-piece cannula in the distal duodenum. Animals were housed in a tie-stall and fed twice a day (8.00 and 20.00h) an equal portion of the daily allowance according to energy and protein requirements. The three lactating cows were fed a diet consisting of 17% corn silage, 23% grass silage and 60% concentrates on dry matter (DM) basis. The dry cow received a mixture of 60% grass silage and 40% corn silage with one kg of concentrates on DM basis.

Samples

Ten feedstuffs were obtained from a parallel feeding experiment: one concentrate, 6 concentrate ingredients and 2 roughages. The concentrate contained the following ingredients: beetpulp (BP, 155 g/kg), maizeglutenfeed (MGF, 300 g/kg), maize feed meal (MFM, 108 g/kg), palmkernel expeller (PKE, 125 g/kg), soyabeanhulls (SBH, 50 g/kg) and soyabeanmeal (SBM, 148 g/kg). The rest of the concentrate was made up with palmkernels (10 g/kg), molasses (80 g/kg) and minerals (24 g/kg). Roughages were grass silage (GS) and maize silage (MS). Samples of concentrate ingredients were obtained from the feed factory before pelleting of the concentrate. Samples from the concentrate,

grass silage and corn silage were taken during the feeding experiment. Concentrate ingredients and the concentrate were ground to pass a 3 mm screen. Roughages were chopped in a laboratory cutter to pieces of approximately 1 cm, and kept at -20°C. Part of the samples was dried at 70°C, ground to pass a 1 mm sieve and used for chemical analysis.

Rumen and intestinal incubations

Nylon bag incubations in rumen and intestine were carried out according to Van Straalen *et al.* (1993). Samples of each feedstuff corresponding to 5 g DM were weighed into nylon bags (9x18 cm, polyamide, pore size 41 um, porosity 30%, Nybolt, Zurich, Switzerland). For each feedstuff 8 bags were incubated for 12 h in the rumen of each cow. The lenght of the incubation time was chosen to have a close agreement between the residue after 12 h incubation and the expected effective escape fraction, based on rumen degradation characteristics of the same feedstuffs (Van Straalen, unpublished results, 1991), and calculated according to Ørskov and McDonald (1979) with a passage rate of 6% per h (Van Straalen *et al.*, 1993). After incubation bags were rinsed under tap water and subsequently washed in a domestic washing machine (using 60 l of water at 15°C without spinning). An extra 4 bags for each feedstuff were not incubated in the rumen and only washed to determine the soluble fractions. Residues were lyophilized, pooled per animal and feedstuff and ground to pass a 3 mm screen. Part of the residues were further ground (1 mm) and used for chemical analysis.

To determine intestinal digestion, nylon bags (3x6 cm, same material as rumen bags) were filled with 0.5 g DM of rumen residue. Pepsin-HCl incubation (1 g pepsin, Merck, Darmstadt, Germany, 2000 FIP-U g, digestion capacity NF XII = 1:10.000, in 1 litre of 0.1 M HCl) was carried out for 1 h at 37°C immediately before intestinal incubation. Bags were introduced in the duodenum (every 20 minutes) via the T-piece cannula (3x4 bags per h) and recovered from the faeces. Recovered bags were rinsed under tap water and stored at -20°C until all bags had been retrieved. Bags were washed in a domestic washing machine, using 100 l of water at 40°C with spinning. This procedure was more intensive than the washing procedure of rumen nylon bags because with the latter method not all washable material may have been removed from the bags (W.M. van Straalen, unpublished results, 1990). Residues were dried at 70°C, pooled per animal and feedstuff and ground to pass a 1 mm sieve.

Laboratory analysis

Feedstuff samples and residues after washing, rumen and intestinal incubation were analyzed for DM, ash and N-Kjeldahl according to the standard procedures at IVVO-DLO (Steg *et al.*, 1990). Residues after washing, rumen and intestinal incubations were pooled per feedstuff before analysis of amino acids. Amino acid analysis was carried out in simplo according to the Proposed Official Method for Determination of Amino Acids in

Animal feed (Dir. 77/101/EEC and 79/373/EEC), as described by Van Vuuren *et al.* (1992). Contents of threonine, serine, valine and isoleucine were corrected for incomplete recovery of amino acids after hydrolysis (Slump, 1969). Non protein-N (NPN) was calculated as total N minus amino acid-N (AAN). The AAN was further separated in essential AAN (EAAN) and non-essential AAN (NEAAN). Feedstuffs were further analyzed for crude fat, starch (Steg *et al.*, 1990), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent insoluble N (ADIN) (Robertson & Van Soest, 1981). Apparent digestibility of organic matter was determined in vitro with rumen fluid followed by pepsin-HCl incubation according to Steg *et al.* (1990).

Comparison of measured and estimated disappearance of N in concentrate

The measured disappearance after washing, rumen and intestinal incubation of the concentrate (Cm) were compared with the values estimated from the disappearance of the ingredients of the concentrate and the respective contents (Ce). Disappearance of palmkernel, molasses and minerals and vitamins were based on tabulated and assumed values.

Statistical analysis

Differences between feedstuffs in rumen and intestinal disappearance of DM and total N were tested using analysis of variance with feedstuff as a factor. Differences between feedstuffs for individual AAN disappearances could not be tested, because samples obtained from four cows had been pooled per feedstuff before analysis of AAN, resulting in only one observation per amino acid and per feedstuff.

For the comparison of the amino acid profile of the protein in the feedstuff, residue after washing, rumen and intestinal incubation, the method of Guilloteau *et al.* (1986) was used. In this method the similarity between two amino acid profiles is expressed in the distance in χ^2:

$$\text{distance in } \chi^2 = 17 \times \sum_{k=1}^{k=17} (AAN_{ik} - AAN_{jk})^2 / ((AAN_{ik} + AAN_{jk})/2)$$

In this equation AAN_{ik} and AAN_{jk} are the respective percentages of N in amino acid k in total AAN of proteins i and j (feedstuff, residue after washing, rumen and intestinal incubation). The same procedure was used to compare the measured and estimated amino acid profiles of the concentrate. A higher distance in χ^2 corresponds with a larger difference between the amino acid profiles of the two proteins. Statistical calculations were carried out using the Genstat software (Genstat 5 Committee, 1987).

Results

Composition of feedstuffs

The chemical composition and composition of total N of samples is given in Table 1 and 2 respectively. Total N content in the samples varied between 12.6 g/kg DM for MS to 82.2 g/kg DM for SBM. The content of ADIN in total N varied from 15 g/kg for SBM to 173 g/kg for PKE. The lowest proportion of AAN in total N was observed for BP (59%) and the highest for SBM (84%). The proportion of EAAN in AAN varied between 45% (MS) and 60% (PKE).

Compared to the other feedstuffs, BP had relative high content of glutamic acid-N, and a low content of arginine-N. PKE had very high proportion of arginine-N in total AAN. Within a class of feedstuffs, amino acid profiles were similar (MGF and MFM; SBH and SBM). Exceptions were the higher glycine-N and lower glutamic acid-N and arginine-N content in SBH compared to SBM. The content of lysine-N and arginine-N in MS was low, and of alanine-N and leucine-N high compared to the other feedstuffs.

Table 1. Content of dry matter (DM), organic matter (OM), N, crude fat, neutral detergent fibre (NDF), acid detergent fibre (ADF), starch and in vitro digestibility coefficient of organic matter (DOM) of feedstuffs (in g/kg DM unless stated otherwise)

	Feedstuff*									
	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM (g/kg)	920	901	899	922	902	892	620	339	906	893
OM	915	939	952	952	949	925	882	954	913	911
N	19,5	34,6	24,6	26,2	22,1	82,2	40,5	12,6	33,4	34,6
Crude fat	7	37	51	103	27	23	43	29	39	40
NDF	300	394	311	640	620	123	450	399	323	330
ADF	148	97	81	368	436	63	239	208	142	13
Starch	nd†	200	335	4	8	8	nd	323	109	98
DOM (%)	89	86	85	70	82	90	79	73	85	85

* BP = beetpulp, MGF = maizegluten feed, MFM = maizefeedmeal, PKE = palmkernel expeller, SBH = soyabeanhulls, SBM = soyabeanmeal, GS = grass silage, MS = maize silage, Cm = concentrate measured, Ce = concentrate estimated from ingredient composition.

† nd = not determined

Table 2. Content of ADIN, NPN, AAN, EAAN, NEAAN and individual amino acid-N in feedstuffs (g/kg N)

	Feedstuff*									
	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
ADIN	47	26	59	173	63	15	22	25	45	41
NPN	411	269	271	204	210	158	400	389	272	232
AAN	589	731	729	796	790	842	600	611	728	768
EAAN	284	378	384	475	406	476	304	278	391	417
NEAAN	305	353	344	321	384	366	296	333	337	351
Thr	25	27	26	22	28	29	27	25	24	27
Val	33	38	35	38	36	37	37	36	35	37
Ile	20	21	21	23	24	31	23	23	24	25
Leu	26	55	55	40	38	50	39	61	47	48
Tyr	19	14	14	12	22	18	12	10	15	16
Phe	12	19	20	20	21	26	21	19	20	21
His	40	52	49	32	52	47	26	34	43	47
Lys	51	36	39	29	73	72	38	25	47	52
Arg	53	108	117	250	107	158	73	35	128	137
Met	6	10	9	10	6	7	7	9	8	8
Asp	43	40	41	49	63	72	55	40	52	54
Ser	37	40	39	37	54	47	33	35	40	42
Glu	116	89	86	107	78	106	49	80	95	99
Pro	26	57	54	28	38	40	54	54	42	44
Gly	38	51	50	50	94	48	45	41	48	50
Ala	37	60	60	39	44	42	52	72	48	48
Cys	7	16	16	10	13	11	6	11	13	13

* for abbreviation see Table 1.

Except for the higher NPN content, the composition of Cm was almost identical to the composition of Ce (Table 1 and 2).

Disappearance after washing

Disappearance of DM after washing varied between 17% for SBH and 45% for Cm (Table 3). The N-disappearance varied between feedstuffs from 11% for SBM to 78% for MS. The NPN showed for all feedstuffs (except SBM) a higher disappearance after washing than AAN. For BP and GS, the EAAN showed a considerable lower value than NEAAN.

The distance in χ^2 indicated a large difference in the amino acid profile of the feedstuff and residue after washing for BP, GS and MS (Table 4). This was mainly due to glutamic acid in BP, proline in GS and arginine in MS (Table 3). For the other feedstuffs washing showed only minor effects on the amino acid profile. In general, threonine and cysteine showed relatively low disappearances, and proline and histidine relatively high disappearances after washing. As regards to the first limiting amino acids, methionine had for most feedstuff a relatively low disappearance, while lysine showed variable results between feedstuffs.

The disappearance after washing of N, NPN and AAN of Ce were lower than of Cm (Table 3). The estimated amino acid profile of the residue was comparable to that of the measured profile; the distance in χ^2 was 4.

Disappearance after rumen incubation

The disappearances after 12 h of rumen incubation of each feedstuff are given in Table 5. Significant differences between disappearance of DM and N were obtained between feedstuffs. The lowest DM disappearance was observed for PKE (39%) and highest for BP (78%). For N the disappearance varied from 24% for PKE to 76% for GS. For PKE the disappearance of AAN was higher than disappearance of NPN, for MGF, MFM and SBH differences were small, and for the remaining feedstuffs the disappearance of AAN was lower than NPN. For BP, PKE, GS, MS and Cm, NEAAN was further degraded in the rumen than EAAN, while differences were small for the other feedstuffs.

The amino acid profile of the residues after rumen incubation were for BP, GS and MS different from that of the feed, but comparable to those after washing, as indicated by the distance in χ^2 (Table 4). In general glutamic acid showed a high disappearance (Table 5). Disappearance of arginine was relatively low for BP and MS, but relatively high for MGF, MFM and SBM. Of the first limiting amino acids, methionine was relatively undegradable for all feedstuffs, especially PKE, while the residual lysine was relatively high in PKE and MS.

The disappearances of total N, NPN and AAN in the rumen of Ce were lower than of Cm (Table 5), but amino acid profiles of rumen residues were comparable (distance in χ^2 was 2).

Table 3. Disappearance after washing of DM, N, NPN, AAN, EAAN, NEAAN and individual amino acid-N in feedstuffs (% of content in feed)

	Feedstuff*									
	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM	44	35	40	19	17	31	35	42	45	41
N	50	48	41	22	31	11	54	78	47	30
NPN	69	60	100	44	80	-2	75	93	81	49
AAN	38	44	18	17	17	14	39	69	35	25
EAAN	22	43	17	17	17	12	33	66	33	23
NEAAN	52	46	19	16	18	15	46	71	37	27
Thr	25	41	8	14	17	6	37	66	26	19
Val	22	38	6	13	9	10	39	66	31	19
Ile	31	37	7	11	12	6	32	71	30	17
Leu	22	40	13	15	16	10	30	74	35	22
Tyr	26	36	5	6	21	10	24	57	26	19
Phe	17	37	9	14	25	8	26	64	29	18
His	29	45	21	28	33	16	50	76	39	29
Lys	24	47	28	13	9	10	36	64	30	21
Arg	10	47	24	19	17	18	28	42	35	26
Met	18	43	21	-15	19	-7	4	63	15	15
Asp	44	45	20	14	27	10	49	68	33	19
Ser	37	50	14	17	19	11	46	70	31	25
Glu	76	46	19	18	24	29	40	74	42	35
Pro	35	46	23	24	18	13	69	77	44	29
Gly	32	41	14	14	9	9	29	61	31	22
Ala	39	46	22	15	7	11	43	76	40	27
Cys	29	43	24	8	37	-2	9	43	30	23

* for abbreviation see Table 1.

Table 4. Distance in χ^2 between AAN profiles of feedstuff (F) residue after washing (S), rumen incubation (R) and intestinal (I) incubation

	Feedstuff*								
	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm
F vs. S	239	7	10	7	16	13	82	119	11
F vs. R	249	20	27	8	44	18	85	226	11
S vs. R	22	11	16	4	35	7	16	71	4
F vs. I	748	668	224	209	474	772	171	586	234
S vs. I	550	634	223	188	442	717	72	703	209
R vs. I	542	617	216	178	262	675	66	677	211

* for abbreviation see Table 1.

Intestinal disappearance

The disappearance of DM and N, expressed as percentage of the rumen residue, differed significantly between feedstuffs and varied from 20% for MS to 88% for SBM and from 64% for MS and SBH to 99% in SBM, respectively (Table 6). The disappearance of NPN varied between 38% for SBH and 97% for SBM, and was for all feedstuffs lower than of AAN, which varied between 70% for SBH and 99% for SBM. EAAN showed a higher disappearance in the intestine than NEAAN.

Judged by the distance in χ^2, intestinal incubation of rumen residues resulted in a considerable change in amino acid profile (Table 4). This difference was highest for MS, SBM, MGF and BP. For most feedstuffs arginine had a relatively high, and glycine and cysteine a relatively low disappearance compared to other amino acids (Table 6). The disappearance of methionine was relatively high in MFM and PKE, while lysine disappearance was relatively low in MGF, MFM, and SBH. In MS an extremely low histidine disappearance was observed.

The intestinal disappearance of the different components of total N of Ce were lower than of Cm, but amino acid profiles were similar (Table 6; distance in χ^2 was 25).

Table 5. Disappearance after 12h rumen incubation of DM, N, NPN, AAN, EAAN, NEAAN and individual amino acid-N in feedstuffs (% of content in feed)

	Feedstuff*									
	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM†	78^{f}	64^{de}	61^{cd}	39^{a}	41^{a}	69^{e}	54^{b}	57^{bc}	69^{e}	66
N†	74^{de}	73^{cd}	61^{b}	24^{a}	62^{b}	59^{b}	76^{e}	74^{de}	69^{c}	62
NPN	89	73	60	18	61	72	83	79	83	69
AAN	63	73	61	25	62	57	72	71	64	60
EAAN	55	72	60	24	63	57	68	66	63	58
NEAAN	71	73	61	27	62	57	76	75	66	63
Thr	60	70	56	19	66	53	73	66	60	58
Val	60	72	59	23	60	52	73	74	63	58
Ile	56	67	52	17	58	52	67	69	59	54
Leu	49	68	51	20	62	54	68	80	62	56
Tyr	66	68	52	14	60	52	67	63	60	56
Phe	49	69	53	22	66	55	70	69	61	56
His	65	78	68	27	59	56	67	73	67	64
Lys	58	71	62	13	57	59	66	40	60	59
Arg	40	76	67	28	69	62	66	41	64	59
Met	45	66	51	1	55	45	60	69	61	49
Asp	63	71	59	24	70	58	77	65	64	64
Ser	68	73	61	25	57	51	75	71	63	59
Glu	85	74	61	32	70	63	72	80	69	67
Pro	57	75	62	24	59	60	86	80	67	64
Gly	57	72	63	26	53	52	70	66	62	58
Ala	62	72	59	22	63	52	74	81	66	61
Cys	55	72	66	21	69	51	60	64	69	60

* for abbreviation see Table 1.

† figures in the same row with different superscripts are significantly different ($p < 0.05$), number of replicates: 4; standard error of difference for DM: 2.4; for N: 3.3 (excluded Ce).

Discussion

Feedstuff composition

The proportion of NPN in concentrate ingredients was generally higher than values given in the Dutch Feedstuff Table (Centraal Veevoederbureau, 1991). Only part of this difference could be attributed to tryptophan, which was not analyzed in our experiment. There were no differences in the ratio EAAN/NEAAN and the profile of individual AA between tabulated values and values measured in our experiment. Only for BP a lower NPN, higher glutamic acid-N and lower aspartic acid-N content was measured compared to Centraal Veevoederbureau (1991). The NPN content in BP increases with the inclusion of molasses. The N in molasses consists for about 70% of NPN and 30% of AAN, which is mainly aspartic acid-N and glutamic acid-N (Centraal Veevoederbureau, 1991). The level of inclusion of molasses in the BP in our experiment was not known, but based on NDF content it was comparable to the quality of BP with the highest sugar content and thus highest inclusion of molasses.

The AA profile of GS observed in our experiment differed with the one published by Rooke *et al.* (1984) and Syrjälä-Qvist *et al.* (1984 a and b). The total and individual EAAN in MS was lower than observed by Le Henaff *et al.* (1988). One can speculate about these differences being due to differences in content of different types of protein as influenced by species, fertilization and maturity.

Solubility

Only for BP a large difference in the soluble N fraction was observed comparing our values to those of Van Straalen and Tamminga (1990), which can be explained by the high level of molasses included. For all feedstuffs (except SBM), NPN was relatively more soluble compared to AAN. Also MacGregor *et al.* (1978) observed a higher solubility of NPN in a modified Burroughs mineral buffer compared to AAN for a large range of feedstuffs. Dependent on the feedstuff, NPN consists of NO_3, NH_3, purine, RNA and DNA, which are relatively soluble or easily degradable in the rumen (Tamminga, 1986; Greife, 1984). The NPN content showed a good relationship with the soluble N (SN) fraction: SN (g/kg N) = -76 + 1.74 x NPN (g/kg N) (r^2 = 0.74; n=9). Besides NPN, also a part of the AAN was washed out from nylon bags. The solubility of total AAN in BP was higher than observed by MacGregor *et al.* (1978), who also reported a higher solubility of glutamic acid compared to the other amino acids. The high solubility of glutamic acid in BP can be explained by the difference in amino acid profile and solubility between the pulp and added molasses as discussed above. For MFM and SBM the solubilities of total AAN were comparable to those of MacGregor *et al.* (1978), but different for individual AAN.

Table 6. Disappearance in the intestine of DM, N, NPN, AAN, EAAN, NEAAN and individual amino acid-N in feedstuffs (% of 12 h rumen residue)

	Feedstuff*									
	BP	MGF	MFM	PKE	SBH	SBM	GS	MS	Cm	Ce
DM†	70^{b}	39^{e}	47^{d}	52^{c}	30^{f}	88^{a}	43de	20^{g}	51^{cd}	52
N†	86^{c}	84^{b}	86^{cd}	85^{cd}	64^{e}	99^{a}	87^{bc}	64^{e}	89^{b}	85
NPN	64	73	83	70	38	97	78	50	71	67
AAN	91	87	87	90	70	99	91	72	92	89
EAAN	92	89	88	91	74	99	91	74	93	91
NEAAN	90	86	85	88	67	99	90	69	91	88
Thr	90	79	79	88	81	100	90	72	90	86
Val	99	88	88	89	74	99	90	71	92	92
Ile	91	89	90	88	82	100	91	75	94	91
Leu	91	90	92	88	83	100	91	75	94	91
Tyr	89	86	88	88	61	99	92	86	91	87
Phe	91	82	90	89	83	100	91	72	94	89
His	86	81	85	88	62	99	89	5	91	85
Lys	93	81	82	90	63	99	92	77	91	88
Arg	93	100	90	94	85	100	92	100	95	94
Met	93	89	92	92	82	100	92	77	93	91
Asp	92	86	87	89	82	100	91	73	94	89
Ser	86	86	86	88	65	99	90	73	91	87
Glu	92	91	92	90	80	100	91	76	95	92
Pro	89	81	85	86	59	99	93	65	89	85
Gly	82	79	79	81	53	98	87	63	84	79
Ala	100	89	77	88	75	99	90	69	92	91
Cys	84	82	82	86	67	99	78	53	88	84

* for abbreviation see Table 1.

† figures in the same row with different superscripts are significantly different ($p < 0.05$), number of replicates: 4; standard error of difference for DM: 3.8; for N: 2.4 (excluded Ce).

Rumen degradation

The residual N after 12 h rumen incubation of most feedstuffs was comparable to the effectively fermented fraction calculated from degradation characteristics in the rumen (Van Straalen, unpublished results, 1991) and an assumed passage rate of 6% per h (respectively 73% for BP, 70% for MGF, 63% for MFM, 45% for PKE, 57% for SBH, 56% for SBM, 78% for GS, 78% for MS, and 67% for Cm). These values were also close to the effective escape N given by Centraal Veevoederbureau (1991). For PKE a higher value was observed, which could be caused by the relative long lag-time before the onset of the degradation, resulting in a large residue after 12 h incubation time in this experiment (A. Steg, unpublished results, 1992).

The additional disappearance of N after rumen incubation (Table 5) compared with after washing (Table 3) varied between feedstuffs. For MS no extra disappearance was measured (-4%), while for SBM another 48% disappeared after rumen incubation. For NPN and AAN this variation was -40% for MFM to 74% in SBM, and 2% for MS and 45% for SBH respectively. From the higher distance in χ^2 between the feedstuff and residue after washing, compared to residue after washing and rumen incubation it can be concluded that depending on the feedstuff the amino acid profile is more influenced by washing than by rumen degradation. This agrees with the conclusion of MacGregor *et al.* (1978) that the amino acid profile of the insoluble residue can be regarded as more representative for that of escape protein than that of the feedstuff.

In our study methionine in SBM was relatively undegradable compared to other amino acids, which agrees with results obtained by Crooker *et al.* (1987), Le Henaff *et al.* (1988) and Mir *et al.* (1984), but disagrees with Susmel *et al.* (1989). The relatively high degradability of arginine and glutamic acid in SBM was also observed by Crooker *et al.* (1986 and 1987). According to literature tyrosine in SBM was relatively undegradable (Ganev *et al.*, 1979; Hennessy *et al.*, 1983; Varvikko *et al.*, 1983), and histidine relatively degradable (Varvikko *et al.*, 1983; Mir *et al.*, 1984), which was not observed in our experiment. The relatively undegradable methionine in GS disagreed with findings of Rooke *et al.* (1984), while the low degradation of proline was similar. Degradation of individual AA in MS were in line with those of Le Henaff *et al.* (1988).

Changes in the amino acid profile after rumen fermentation were mainly observed with BP and MS (Table 4). Because total N content of BP and MS is low, microbial contamination of rumen residues can have a large effect on the amino acid profile of the residues. The high residues of lysine and arginine in both BP and MS are an indication that these residues contained microbial protein, which has a relative high content of those amino acids (Rooke *et al.*, 1984). Diaminopimelic acid (DAPA) was used to estimate microbial contamination. However, the DAPA content of both feedstuffs and residues was generally below the detection level of 0.50 g/kg DM, and if it was higher a large variation was observed. The DAPA content was therefore assumed to be unsuited for estimation of the microbial contamination. An attempt was made to estimate microbial contamination according to Van Bruchem *et al.* (1985), by means of comparison of amino

acid profiles of the feed, microbial protein (Rooke *et al.*, 1984) and rumen residue. The method of Van Bruchem *et al.* (1985) incorrectly assumes that the amino acid profile of feed does not change during rumen incubation, and therefore the amino acid profile of the insoluble residue was used instead that of the feed. Estimated microbial contaminations were 27%, 0%, 10%, 4%, 0%, 5%, 12%, 36% and 0% of total AAN for BP, MGF, MFM, PKE, SBH, SBM, GS, MS and Cm respectively. Estimated contaminations agreed with those observed for concentrate ingredients (Crooker *et al.*, 1986 and 1987) but were lower for roughages (Nocek and Grant, 1985; Messman *et al.*, 1992). If the amino acid profile of the feed was used, estimated contaminations for all feedstuffs were higher, especially for BP, GS and MS: 78%, 53% and 58% respectively. Those feedstuffs also showed high differences between the amino acid profile of the feedstuff and residue after washing. This indicates the importance of using the amino acid profile of the insoluble residue rather than that of the feed. It was concluded that microbial contamination can not be neglected for at least BP, GS and MS.

Besides differences in solubility and microbial contamination, changes in the amino acid profile after rumen incubation can also be caused by different degradation rates between individual amino acids. Messman *et al.* (1992) observed only small differences between individual amino acid degradation rate with bromegrass hay, while with grass silage, Rooke *et al.* (1984) found consistent differences in degradation rates between individual AAN. Differences in degradation rate of individual amino acids might be dependent on the content of the different protein classes (albumin, globulin e.a.) in the feedstuff and their respective physical properties (solubility, structure) and amino acid composition (Messman *et al.*, 1992).

Intestinal disappearance

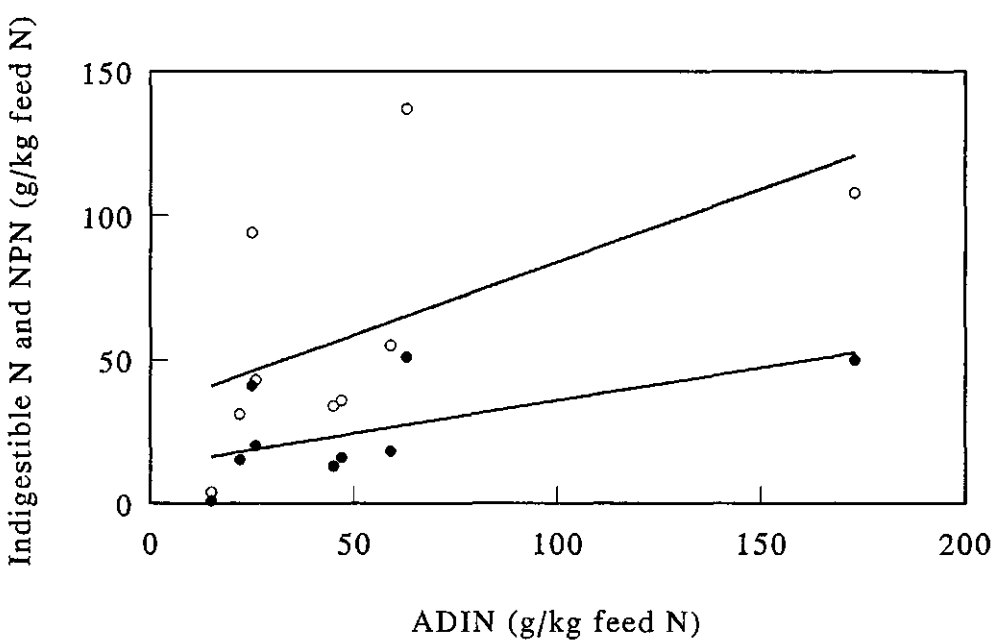
Because mobile nylon bags were recovered from the faeces, the disappearance of N is the sum of small intestine and large intestine disappearance. Although the latter was not measured in our experiment, other studies indicate that large intestine fermentation has only a limited effect on total intestinal disappearance, both in nylon bag (Hvelplund, 1985; Voigt *et al.*, 1985) or in *in vivo* experiments (Van Straalen and Tamminga, 1990).

Total N disappearance from mobile nylon bags of concentrate ingredients were similar to the digestibility coefficients in the small intestine mentioned by Centraal Veevoederbureau (1991). The disappearance of total AAN in the intestine was higher than of total N and showed a close relationship: disappearance of AAN (g/g) = 0.18 + 0.82 * disappearance of total N (g/g) (r^2 = 0.98; n=9). With this equation data from grass silage and hay of Varvikko and Vanhatalo (1991) could be reasonable fitted (r^2 between predicted and measured disappearance of AAN was 0.77). If this relationship also accounts for other feedstuffs, it can be used to predict the AAN disappearance from total N disappearance in mobile nylon bags.

The disappearance of NPN was generally lower than the intestinal digestibility of nucleic acids as mentioned by Greife *et al.* (1984). This finding indicates that NPN in

rumen undegraded N is mainly linked to components that are indigestible in the intestine. Combined with the observations of NPN disappearance in the rumen, this leads to the conclusion that NPN in feedstuffs consists of two fractions: one that is highly soluble in the rumen, and one that is linked to cell walls and is indigestible. In agreement, Hof *et al.* (1990) observed a decrease of NPN in rumen residues at short incubation times and increase at long incubation times. As measure of indigestible N the ADIN content was proposed (Robertson and Van Soest, 1981). However, the relationships between the indigestible N or indigestible NPN measured with mobile nylon bags on one hand and the ADIN content on the other hand were poor (Figure 1). Webster *et al.* (1984) proposed to estimate the small intestinal digestibility of total N (dUN) from ADIN and undegraded dietary N (UDN): dUN = 0.9 x (UDN-ADIN)/UDN. This approach was adopted in the Metabolisable Protein system (AFRC, 1992). There was no relationship between dUN calculated for feedstuffs in our experiment (from Table 2 and 5) and the disappearance of total N from nylon bags in the intestine (Table 6) (r^2 = 0.19). For most samples the disappearance of N from mobile nylon bags was higher than dUN (except for SBH and MS). Using mobile nylon bag data will therefore result in higher predicted supply of absorbable feed N compared to the approach of Webster *et al.* (1984). This conclusion agrees with the comparison of these methods that can be carried out using data from other feedstuffs as presented by AFRC (1992).

Figure 1. Relationship between indigestible N (○), indigestible NPN (●) measured with nylon bag incubations and ADIN (all expressed as g/kg feed-N). Lines represent regression equations: IN = 33.5 + 0.505 x ADIN (r^2=0.32); NPN = 12.8 + 0.230 x ADIN (r^2=0.39)



Intestinal incubation showed for all feedstuffs a considerable effect on the amino acid profile. For most of the feedstuffs this could be explained by the relatively high disappearance of arginine and low disappearance of glycine. Therefore, these amino acids accounted for the difference between the intestinal disappearance of EAAN and NEAAN. The high disappearance of arginine could be the result of the action of trypsin, which is an endopeptidase that hydrolyses only lysine and arginine-bonds (Stryer, 1988). Le Henaff *et al.* (1988) however, did not find a higher arginine disappearance in the intestine. The theory also doesn't explain the variable disappearances obtained for lysine. The low disappearance of glycine might be an indication of contamination with endogenous protein which has a high content of glycine (Laplace *et al.*, 1985).

Predicted supply of absorbable feed amino acids to the animal

From the disappearance of individual AAN from nylon bags in the rumen (Table 4) and intestine (Table 6), the supply to the animal of individual amino acid-N from the feed that is absorbed in the small intestine can be predicted. It was assumed that the residue after rumen incubation was representative for the escape fraction and all amino acid-N that disappeared from the mobile nylon bags was absorbed in the small intestine. The predicted supply of AAN (expressed as percent of AAN in the feed) varied between feedstuffs from 21 to 67% and was for all feedstuffs higher than the supply of NPN (7% to 57%). Predicted supply of EAAN (25 to 69%) was higher than supply of NEAAN (17 to 64%). The predicted supply of methionine was higher than of other amino acids for all feedstuffs but CS. For BP, GS and CS lysine had the second highest supply. Also isoleucine, leucine, tyrosine and arginine had a high supply for several feedstuffs. Of the EAAN histidine showed the lowest supply for MGF, MFM and CS. For most feedstuffs the supply of glutamic acid and proline was lowest. Also other NEAAN showed poor supply compared to EAAN.

Measured and estimated AAN fermentation and digestion of concentrate

No explanation can be found for the higher AAN content of Ce compared to Cm. The lower disappearance after rumen incubation of all N fractions of Ce compared to Cm was mainly due to the lower SN fraction. A reason for this can be the treatment (grinding, mixing and pelleting) of concentrate ingredients during the manufacturing process of the concentrate, which might have reduced particles sizes, resulting in a higher disappearance of N from nylon bags in the washing machine. Results are in contrast to those of Murphy and Kennelly (1987), who observed similar measured and estimated protein degradation in the rumen, which can be explained by the absence of pelleting of concentrate mixtures. In contrast to the rumen residues, intestinal disappearance of all N fractions in concentrate could be estimated from the values obtained with ingredients. Because of the higher rumen residues, the estimated absorption of individual amino acid-N were higher than the measured values. The amino acid profile of undegraded AAN or small intestine digestible

AAN in concentrates could be very well estimated from that of the individual concentrate ingredients.

Conclusions

Although differences between feedstuffs do exist, it can be concluded that AAN was less degradable in the rumen than NPN, and that EAAN was less degradable than NEAAN. Dependent on the feedstuff, rumen incubation had a pronounced effect on the amino acid profile, which was mainly due to difference in solubility of amino acids. Intestinal incubation resulted in a considerable change in amino acid profile of the indigestible residue. It can be concluded that feed protein that is assumed to be absorbed from the small intestine is higher in EAAN, especially methionine-N and lysine-N, and lower in NEAAN, especially glutamine-N and proline-N, than protein in the feedstuff.

Acknowledgements

The authors are grateful to S. Tamminga (Agricultural University Wageningen) and A.M. van Vuuren for their contribution to the discussion and critically reading the manuscript. We also highly appreciate assistance of R. Terluin and his team during the experiment. This study was financed partly by Financieringsoverleg Mest- en Ammoniak-onderzoek (Fund for Manure and Ammonia Research).

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CHAPTER 7

DEVELOPMENT AND VALIDATION OF A MODEL TO DESCRIBE NITROGEN FLOW IN DAIRY COWS

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Summary

To enable reduction of N-losses in dairy cows, a model to predict N-flow was developed and validated. The N-flow model described the fermentation and digestion of amino acid-N (AAN), non-protein-N (NPN), lipids (LI), neutral detergent fibre (NDF), sugar and starch (SS), fermentation products (FP) and miscellaneous compounds (MC) in the rumen, small intestine and large intestine; and fate of AAN and NPN in the metabolism. Fermentation and microbial growth in the rumen and large intestine were dependent on fractional passage rates measured by marker methods and degradation characteristics measured by nylon bag incubations. For some parameters (mainly for LI and MC) data were lacking, so assumptions were used. Intestinal digestion and metabolic processes were described by coefficients. A sensitivity analysis was performed to test the effect of parameter assumptions on N-flows. Validation was carried out with data from duodenal and ileal flow measurements, N-balance and feeding experiments.

Predicted flows and excretions were very sensitive to the digestibility coefficients in the small intestine of escape feed, microbial and endogenous AAN and NPN, and endogenous contribution of CP in the small intestine. Fractional passage and degradation rates showed only a minor effect on flows and excretions, with the exception of the fractional degradation rate of feedstuffs in the rumen.

The N-flow model could reasonably predict total duodenal N flow, but underestimated the microbial contribution, probably due to inadequate description of NDF fermentation or underestimation of the degradation rate. To obtain a close agreement between predicted and observed AAN and NPN flow in the ileum, negative digestibility coefficients of endogenous AAN and NPN were needed.

The N-flow model showed a slight overestimation of faecal N-excretion. The microbial contribution to faecal N was low compared to literature values indicating that fermentation of OM in the large intestine was underestimated. Also urinary N-excretion was slightly overestimated. The main and most variable source of urinary-N was N lost in the rumen. Milk N-excretion could be accurately predicted due to a variable efficiency of milk protein production that was used in the N-flow model.

The N-flow model can be used to further study the possibilities of feeding management to reduce N loss in dairy cows.

Introduction

In The Netherlands an increasing awareness of the negative impact of intensive dairy husbandry on the environment has developed in recent years. Dairy husbandry is held responsible for a major part of the N-excretion to the environment, assumed to contribute to acidification of air and soil (NH_3) and pollution of ground water (NO_3). Of the total N intake of an average dairy cow in The Netherlands, it was estimated that 29% was excreted in faeces, 50% in urine, 19% in milk, and 2% deposited in body reserves (Tamminga, 1992). Part of the N excreted in faeces and urine is due to (lack of) digestion and metabolic processes and therefore considered inevitable. Another part is due to an imbalance between the supply and what the tissues and organs can efficiently handle. Reductions in N excretion can mainly be achieved by lowering total N intake and balancing N and energy supply on both rumen and metabolism level (Tamminga, 1992). Although modern protein evaluation systems (NRC, 1985; Vérité *et al.*, 1987, Tamminga *et al.*, 1994) can be used to estimate milk N excretion, they can not directly be used to estimate N losses in faeces and urine. Furthermore, these systems use a rather static approach which is not adequate to describe the N flow in the animal.

In this study recently acquired knowledge about protein degradation and digestion in the gastro-intestinal tract and metabolism is integrated into a model. The aim of this N-flow model is to predict the flow of N through the gastro-intestinal tract and excretion of N in milk, urine and faeces, in order to prevent unnecessary loss of N in dairy cows.

At different levels of the digestion and metabolic processes in the animal energy and protein interact. To predict N-flow through the gastro-intestinal tract, the N-flow model therefore needs to describe the fermentation and digestion of all components of the organic matter (OM). At metabolic level the Dutch NEL-system (VEM-system) was used as measure of energy.

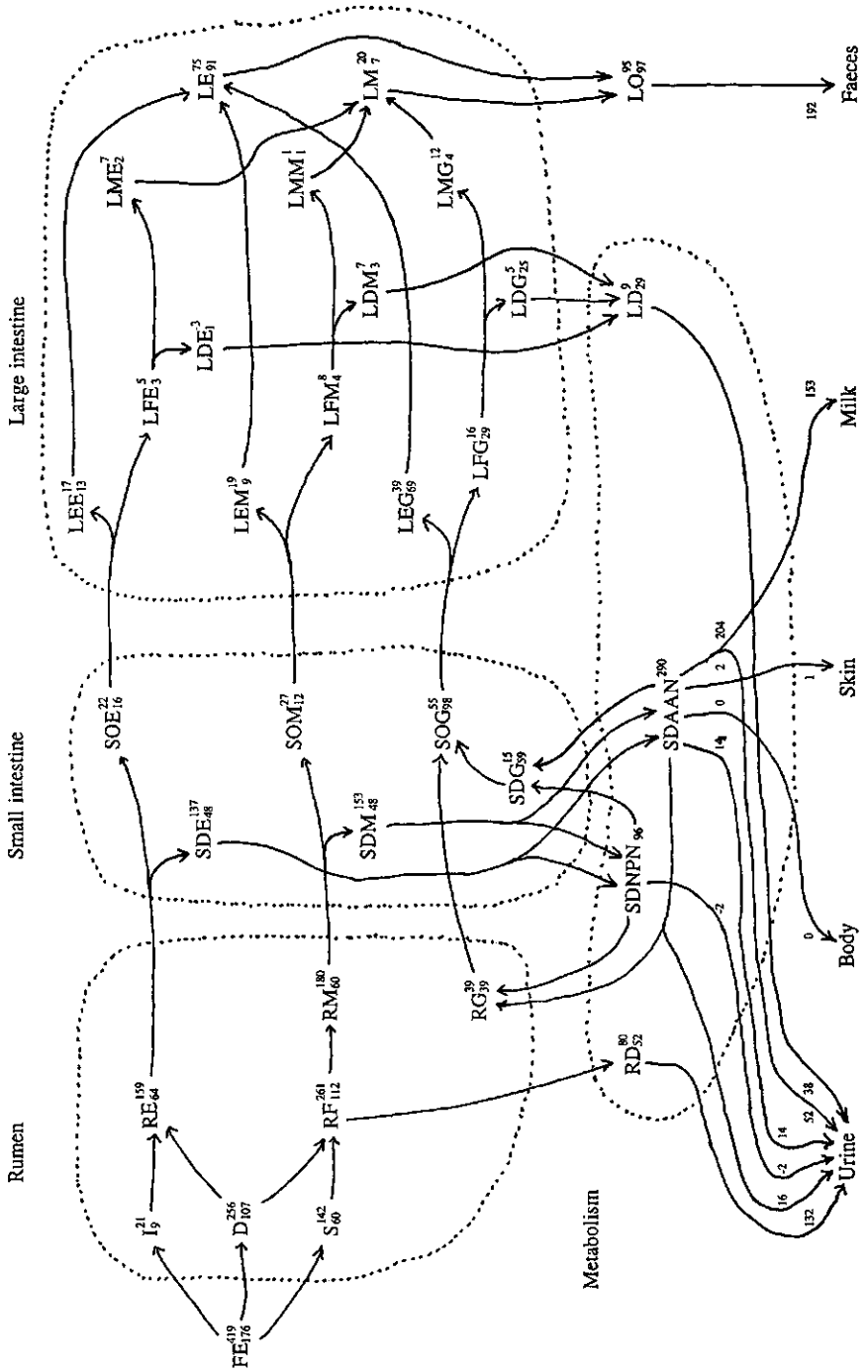
The coefficients and formulas in the N-flow model were mainly based on data from literature and research carried out at ID-DLO. However, during validation it became apparent that some parts of the N-flow model needed further adaption to improve the prediction of N excretions. These adaptions are only briefly addressed in the section on the description of the N-flow model, while the background is further explained in the discussion section.

Description of the N-flow model

Model outline

The N-flow model consists of four compartments: 1: rumen; 2: small intestine; 3: large intestine and 4: metabolism. The input and statements in the N-flow model, and an explanation of the abbreviations are given in the Appendix. Figure 1 shows a schematic

Figure 1. Schematic presentation of the N-flow model. Figures represent AAN (superscripts) and NPN-flow (subscripts) in g/d on the standard diet used in the sensitivity analysis



presentation of the N-flow model. The data in Figure 1 represent the amino acid-N (AAN) and non-protein-N (NPN) flow in the standard diet of the sensitivity analysis (see later). The gastro-intestinal compartments describe the fate of total OM for each individual feedstuff. The metabolism compartment describes total dietary AAN and NPN.

Feed OM that enters the rumen is either fermented and used for microbial production, or escapes rumen fermentation. Fermented OM that is not used for microbial production is digested and absorbed through the rumen wall and enters the metabolism compartment. The rumen is assumed to be a total mixing compartment: the balance between fermentation and escape depends on fractional degradation and passage rates of different fractions of the feed. The efficiency of microbial synthesis depends on fractional degradation rates.

The output from the rumen by passage and subsequent input into the small intestine compartment of OM consists of rumen microbes, escaped feed and endogenous excretions. All these fractions are partly digested in the small intestine and enter the metabolism compartment. The small intestine is assumed to be a tubular compartment in which digestion is determined by digestibility coefficients.

The large intestine is comparable to the rumen. The OM that is not digested in the small intestine is again subjected to microbial fermentation. The balance between large intestine fermentation and escape depends on fractional degradation and passage rates of different fractions. Fermented OM that is not used for microbial synthesis is absorbed by the large intestine wall into the metabolism compartment. Faecal excretion consists of large intestine escape and microbial components. To enable comparison of predicted flows with observed flows, the predicted output from the rumen compartment is comparable to the observed flow at the end of the duodenum, inclusive endogenous excretion in the duodenum (further referred to as duodenal flow). The predicted output of the small intestine is comparable to observed flow at the end of the ileum (ileal flow).

The N-input into the metabolism compartment consists of N absorbed in the rumen (only non-protein-N; NPN), small intestine (AAN and NPN) and large intestine (only NPN). Digested AAN is primarily used for maintenance: endogenous urinary-N, endogenous gastro-intestinal-N, and N lost in skin and deposited in body reserves. The remaining AAN is used for the production of milk protein-N. All these processes have their specific efficiency. The AAN not efficiently used and NPN digested from the gastrointestinal tract are excreted in the urine.

Throughout the paper abbreviations with a subscript denote a fraction or coefficient: e.g. S_{CP} = solubility of CP (g/g) and SD_{AAN}=small intestine digestibility of AAN (g/g). Abbreviation without subscripts are used for flows: e.g. SDAAN = small intestine digested AAN flow (g/d). Equations numbers in the text correspond with those in the Appendix.

Model input and parameters

Division of organic matter

In the N-flow model, OM of each feedstuff was separated in the following components: crude protein (CP), lipids (LI), neutral dergent fibre (NDF), starch and sugar (SS), fermentation products (in ensiled feedstuffs, FP) and miscellaneous components (MC). If the NDF or SS content of roughages in de datasets used for development and validation of the N-flow model, was not available values were estimated from the contents of other components (Table 1). The FP in grass silage and corn silage were estimated according to Steg *et al.* (1991). The MC content of feedstuffs was calculated as: OM - CP - LI - NDF - SS - FP. Flow of OM was calculated as the sum of CP, LI, NDF, SS, FP and MC flow. CP flow was calculated as the sum of N x 6.25 and NPN x 6.25.

Division of components of OM

Each component of the OM (i = CP, LI, NDF, SS and MC) was divided into a directly soluble fraction (S_i), a total tract indigestible fraction (I_i) and a potentially degradable fraction ($D_i = 1 - S_i - I_i$). The S-fraction was defined as the fraction of each component that disappears after washing the feedstuff in nylon bags in a domestic washing machine (Van Vuuren *et al.*, 1989). Fraction I was in case of NDF defined as the fraction that is still present in nylon bags after 14d of rumen incubation (Robinson *et al.*, 1986). For CP this fraction was the residue after incubation of feedstuffs in the rumen and intestine, determined by means of the mobile nylon bag technique (Van Straalen *et al.*, 1993). It was further assumed that LI was not degraded in the rumen (degradation rate = 0) and had an I-fraction of 0.15, and that FP were completely soluble. No information was available on the degradation characteristics of the MC. Based on data obtained for non-starch polysacharides the S-fraction was set to 0.10 (Lonkhuijsen and Cone, 1992) The I-fraction of MC was assumed to be 0.15.

For concentrate ingredients, fractions of CP, SS and NDF were taken from Tamminga *et al.* (1990) and Van Straalen and Tamminga (1990), incidentally supplemented with other literature data. For roughages these fractions were estimated using equations based on data from experiments carried out at ID-DLO and Wageningen Agricultural University (for references see Table 2). Relationships between chemical composition (DM, CP and NDF) and fractions (S and I) of CP, NDF and SS were obtained using multiple regression analysis. When the variance accounted for was lower than 50 percent, the average was used as an estimate. Average fractions and equations to estimate fractions are given in Table 1.

Table 1. Averages and equations (e1 to e13) to predict NDF and SS content and degradation characteristics of CP, NDF and SS of roughages[1]

Roughage	S_{CP} (g/kg CP)	I_{CP} (g/kg CP)	$kd1_{CP}$ (%/h)	NDF (g/kg DM)	I_{NDF} (g/kg NDF)	$kd1_{NDF}$ (%/h)	SS (g/kg DM)	S_{SS} (g/kg SS)	I_{SS} (g/kg SS)	$kd1_{SS}$ (%/h)
Grass	140	50	10.1	e1	110	e2	-	-	-	-
Grass silage	e3	e4	6.5	e5	190	3.5	-	-	-	-
Grass hay	340	114	4.4	e6	e7	3.2	-	-	-	-
Clover	e8	e9	7.5	e10	e11	4.4	-	-	-	-
Corn silage	570	90	2.3	e12	320	1.9	e13	520	10	8.3

Roughage	nr.	Equation	R^2	n
Grass	e1	NDF = -99.6 +0.535 x DM + 2.002 x CF	0.82	50
	e2	kd_{NDF} = 0.034 x CP - 0.004 x NDF	0.54	72
Grass silage	e3	S_{CP} = 892 - 0.337 x DM - 0.320 x NDF	0.51	42
	e4	I_{CP} = 0.115 x NDF	no equation	
	e5	NDF =269.1 + 0.163 x DM - 0.546 x CP + 0.968 x CF	0.94	40
Grass hay	e6	NDF = 128.4 + 1.616 x CF	0.96	11
	e7	I_{ND} = 0.442 x NDF	0.60	7
Clover	e8	S_{CP} = 463 - 1.806 x DM	0.54	8
	e9	I_{CP} = 0.331x NDF	0.77	8
	e10	NDF = -263.4 + 0.998 x DM + 2.567 x CF	0.87	7
	e11	I_{NDF} = -131 + 1.114 x NDF	0.89	8
Corn silage	e12	NDF = 60.9 + 1.866 x CP + 0.974 x CF	0.92	6
	e13	SS = 532.9 +0.471 x DM - 0.945 x CF	0.88	24

[1] Data from: Bosch, 1991; Hageman *et al.*, unpublished; Tamminga *et al.*, 1992; Steg *et al.*, 1994; Van Vuuren *et al.*, 1990; and unpublished

Fractional degradation and digestion rates

Rumen degradation of the S_i and D_i-fractions of each OM component was assumed to occur according to a first order kinetic function (Ørskov and McDonald, 1979):

$$residue_t = D_i \times e^{(-kd1i \times t)} + S_i \times e^{(-kd1s \times t)};$$

with: $kd1_i$ = degradation rate in the rumen of D_i-fraction (/h);
$kd1s$ = degradation rate in the rumen of S_i-fraction (/h)

For all components the S_i-fraction was assumed to be instantly degraded in the rumen: $kd1s$ = 3.0 /h (Russell *et al.*, 1992). The $kd1_i$ of CP, NDF and SS were based on nylon bag incubations in the rumen, and obtained for concentrates and roughages as previously described for S_i and I_i fractions. Fractional degradation rates of D_i-fractions of LI and MC were arbitrarily set to 0.0 and 0.05 (/h) respectively.

The digestibilities of components in the small intestine were assumed to be constant, except for the escape feed-N (Table 2). Further explanation is given in the section of description of the small intestine.

Table 2. Digestibility coefficients in the small intestine of escape feed (SDE_i), microbial and endogenous components

Source	Component N	AAN	NPN	LI	NDF	SS	MC
Escape feed	[1]	[2]	[3]	0.85	0.00	[4]	[5]
Microbial[6]	0.84	0.85	0.80	0.85	0.00	-	-
Endogenous	-0.94	-0.39	-1.49	-	-	-	

[1] $SDE_N = (RE_N - U_N) / RE_N$; with $U_N = I_N / [\ kp3/(kd3+kp3)]$; (eq. 2.1a and 2.1b)
[2] $SDE_{AAN} = 0.18 + 0.82 \times SDE_N$ (Van Straalen *et al.*, 1994); (eq. 2.2)
[3] $SDE_{NPN} = (SDE_N - SDE_{AAN} \times AAN) / NPN$; (eq. 2.3)
[4] $SDE_{SS} = -0.728 \times RE_{SS} + 0.879$ (Nocek & Tamminga, 1991); (eq. 2.4)
[5] SDE_{MC} calculated as for N;
[6] from: Hvelplund (1985); Greife (1984); Storm *et al.* (1983);

The degradation in the large intestine was assumed analogous to that in the rumen. Fractional degradation rates of feed components ($kd3_i$) were set to half of that $kd1_i$ (Appendix eq. 0.1), based on the difference in fermentation pattern between the rumen and large intestine (Demeyer & De Graeve, 1991), and a comparison of *in vitro* methods with rumen fluid and faecal suspensions (Aiple *et al.*, 1992). The fractional degradation rate of microbial and endogenous components were both assumed to be 0.05 /h.

Fractional passage rates

Passage of OM components in the gastro-intestinal tract was distinguished in passage of fluid (kpf; only in the rumen), roughage particles and concentrate particles. In the N-flow model, passage from the rumen (kp1) and large intestine (kp3) were assumed to occur according to first order kinetics. Passage through the small intestine was described by a transit time (TT2). Total tract mean retention time (TMRT) could then be calculated according to the model of Grovum and Williams (1979):

TMRT = 1/kp1 + TT2 + 1/kp3

with:

TMRT = total tract mean retention time (h);
kp1 = fractional passage rate in the rumen (compartment 1) (/h);
TT2 = transit time in small intestine (compartment 2) (h);
kp3 = fractional passage rate in the large intestine (compartment 3) (/h).

A literature study was carried out to obtain values of kpf and kp1, kp3, TT and TMRT of particles in dairy cows measured by different markers, and estimated using the model of Grovum and Williams (1979). Passage characteristics were related to DM-intake and proportion of roughage in the diet. The best relationships were obtained using data from experiments in which passage of fluid was measured with Co-EDTA and passage of roughage particles and concentrate particles were measured with rare earth metals (Table 3; eq. 0.2 to 0.5b).

Rumen NDF outflow based on fractional passage rates measured with external markers and fractional degradation rates measured with nylon bag incubations were shown to be unreliable (Tamminga *et al.*, 1989). To estimate rumen fractional passage rates of NDF therefore a different approach was followed. The proportion of the D-fraction of NDF fermented in the rumen was assumed to be constant at 0.80 (see Discussion), which implies that: $kp1_{NDF} = 0.25 \times kd1_{NDF}$ (eq. 0.4a and 0.4b).

No relationships were observed ($R^2 < 0.50$) between DM-intake or proportion of roughage in the diet and kp3 and TT2. Fractional passage rate in the large intestine was therefore calculated from TMRT and kp1 by adding TT2 and 1/kp3 to total intestinal TT. It was assumed that large intestine transit time (1/kp3) was 0.4 of total intestinal TT, reflecting the ratio between the volumes of the small and large intestine (O'Connor *et al.*, 1984) (eq. 0.6a and 0.6b). In the large intestine, NDF was assumed to have an equal fractional passage rate as total OM (eq. 0.7a and 0.7b).

Table 3. Mean and range (between brackets) of DM-intake (KGDM), proportion of roughage in the diet (%R), rumen passage rates of fluid, concentrate and roughage particles and TMRT in the gastrointestinal tract of dairy cows from literature data used to establish regression equations

Item	DM-intake (kg/d)	Roughage (g/g DM)	kp1 (/h)	TMRT (h)	n
Fluid	17.7	0.56	0.115		64[1]
	(5.4-28.3)	(0.27-1.00)	(0.055-0.153)		
Roughage	18.4	0.53	0.045	44.4	19[2]
	(7.2-25.7)	(0.28-0.83)	(0.023-0.064)	(31.0-78.1)	
Concentrate	19.3	0.53	0.064	36.3	20[2]
	(7.4-25.7)	(0.28-0.83)	(0.038-0.112)	(23.7-51.6)	

Passage	Equation		R^2	Equation
Fluid	kp1f	= (-3.40+1.224xKGDM-0.030xKGDM^2+5.93x%R)/100	0.41	0.2
Roughage	kp1r	= (1.743 + 0.149xKGDM)/100	0.55	0.3a
Concentrates	kp1c	= (10.08 - 0.963xKGDM + 0.037xKGDM^2)/100	0.55	0.3b
Roughage	TMRTR	= 71.84 - 1.59 x KGDM	0.57	0.4a
Concentrates	TMRTC	= 57.65 - 1.110 x KGDM	0.52	0.4b

[1] references: Bosch (1991), Cameron *et al.* (1990), Cameron *et al.* (1991a), Cameron *et al.* (1991b), Johnson and Combs (1989), Klusmeyer *et al.* (1991), Lindberg (1982), Robinson and Sniffen (1985), Robinson and Kennelly (1991), Sarwar *et al.* (1991), Snyder *et al.* (1984), Van Vuuren *et al.* (1991), Woodford and Murphy (1988);

[2] references: Colucci *et al.* (1982), Hartnell and Satter (1979), Shaver *et al.* (1986), Stern *et al.* (1983), Weiss *et al.* (1989), Woodford *et al.* (1986), Van Vuuren *et al.*, unpublished.

Model calculations

Rumen

The extent of rumen fermentation of each component was dependent on the fractions S_i and D_i, and the ratios between kpf and kd1s for the S_i-fractions and kpl (concentrate and roughage separately) and $kd1_i$ for the D_i-fraction (Ørskov and McDonald, 1979). Not fermented components were assumed to escape from rumen degradation (eq. 1.1a to 1.6).

For the estimation of microbial growth in the rumen the approach of the Cornell Net Carbohydrate and Protein System (CNCPS) was adapted (Russell *et al.*, 1992). In this approach two groups of microorganisms were distinguished: NSC-microbes using non-structural carbohydrates (SS, FP and MC) as substrate and SC-microbes using structural carbohydrates (NDF). The efficiency of microbial synthesis on each substrate (YM) was assumed to depend on the degradation rate of each substrate (= growth rate; kd in %/h), maintenance requirements (Ymain in g substrate/g microbes/h) and maximum growth rate (Ymax in g microbes/g substrate) (Russell *et al.*, 1992):

$$YM = 1 / (Ymain/kd + 1/Ymax)$$

The Ymain was assumed to be 0.15 for NSC-microbes and 0.05 for SC-microbes; the Ymax was similar for both groups (0.33) (Russell *et al.*, 1992). In our N-flow model also NSC-microbial synthesis from fermented CP was included, with YM half of that of non-structural carbohydrates (Demeyer and Tamminga, 1987) (eq. 1.7 to 1.22). Rumen microbial synthesis for each group of microbes was calculated from the efficiency and available substrate (eq. 1.23 to 1.27). The DM-composition of microbes was calculated from literature values summarized by Dijkstra *et al.* (1993). The NSC-microbes contained 12% ash, 63% CP, 13% LI, 2% NDF and 10% MC, and the SC-microbes 13% ash, 70% CP, 14% LI and 3% NDF.

Rumen digested feed components were calculated as the difference between rumen fermentation and microbial synthesis (eq. 1.28 and 1.29).

From data presented by Brandt *et al.* (1980) and Ørskov and MacLeod (1982) it was calculated that the flow of endogenous CP in the proximal duodenum was 0.03 g/g OM passing in this section. In the distal duodenum this value was doubled (Brandt *et al.*, 1986; Van 't Klooster and Rogers, 1969; Voigt *et al.*, 1993). Total endogenous contribution of CP was then 0.06 g/g passing OM. Because no data were available the proportion AAN in total endogenous N was arbitrarily set to 0.5. Based on the predicted MC flow in the small intestine, it was further assumed that 0.18 g of endogenous MC is excreted per g passing OM (see Discussion). The endogenous flow of each component at the end of the duodenum was then calculated by multiplying those figures with the predicted OM flow (exclusive endogenous) to the small intestine (eq. 1.30 to 1.34).

The total output of each component from the rumen compartment into the small intestine compartment was the sum of escape, microbial and endogenous components (eq. 1.35 and 1.36). The rumen N balance was calculated as the difference between fermented and microbial N (eq. 1.37).

Small intestine

The components flowing into the small intestine are all subjected to enzymatic digestion in the small intestine. Digestibility coefficients of escape feed (SDE_i) were

assumed to be constant for all components, except for N (Table 2). The latter was calculated from the escape fraction (RE_N), and the indigestible residue after rumen and intestinal incubation by means of the mobile nylon bag technique (Van Straalen *et al.*, 1993), corrected for loss of N from mobile nylon bags in the large intestine due to microbial fermentation. The rumen escape feed-N fermented in the large intestine was assumed to be dependent on fractional passage and degradation rates in the large intestine. The residue after mobile nylon bag incubation (I_N) then showed the following relationship with the output from the small intestine (U_N):

$I_N = U_N \times [kp3/(kd3_N+kp3)]$; which can be rewritten as:

$U_N = I_N \times [(kd3_N+kp3)/kp3]$.

The digestibility coefficient of escape feed N (SDE_N) in the small intestine was calculated as:

$$SDE_N = (RE_N - U_N) / RE_N \qquad \text{(eq. 2.1a and 2.1b)}$$

From the digestibility coefficient of total N, those of AAN (SDE_{AAN}) and NPN (SDE_{NPN}) can be calculated as follows:

$$SDE_N \times REN = SDE_{AAN} \times REAAN + SDE_{NPN} \times RENPN$$

The AAN and NPN content of escape feed were assumed to be equal to that of the feed, and taken from CVB (1991). The SDE_{AAN} was higher than that of total N and showed the following relationship (Van Straalen *et al.*, 1994b):

$$SDE_{AAN} = 0.18 + 0.82 \times SDE_N \qquad \text{(eq. 2.2)}$$

In the N-flow model, SDE_{AAN} and SDE_{NPN} were calculated from SDE_N and AAN and NPN content in the feedstuff using the above mentioned equations (eq. 2.2 and 2.3).

Digestibility coefficients in the small intestine of microbial AAN and NPN were fixed at 0.85 and 0.80 respectively (Hvelplund, 1985; Greife, 1984; Storm *et al.*, 1983).

Digestibility coefficients of total endogenous N reported in literature varied between 0.50 (Voigt *et al.*, 1993) and 0.85 (Krawilitzki *et al.*, 1990). No data on the digestibility of endogenous AAN or NPN were available, but initially those of total N were used. However, during validation it showed that using the above mentioned coefficients, the flow of total N at the end of the ileum was highly underestimated. It was therefore decided to follow a different approach for the estimation of the digestibility coefficients of endogenous AAN and NPN. For this approach measurements of N flow at the end of the ileum with cannulated animals were used (10 treatments, Südekum *et al.*, 1989). This

dataset was supplemented with ileal N flows estimated from faecal N excretion observed by Tamminga *et al.* (unpublished). Ileal N flow for the latter data was calculated according to a relationship observed in the data of Südekum (1989): ileal N-flow = 24.3 + 1.05 x faecal N flow (R^2 = 0.99). Because the flow of AAN or NPN was not given, the ratio of AAN in total ileal N was fixed at 0.45 (Tamminga, 1975). The flow of endogenous AAN and NPN in the ileum was subsequently calculated as the difference between the total AAN and NPN flow as previously described, minus the flow of escape feed and rumen microbial AAN and NPN not digested in the small intestine, that was predicted by the N-flow model. The digestibility coefficients of endogenous AAN and NPN were calculated using regression analysis of the predicted endogenous AAN and NPN flow in the duodenum on the calculated endogenous AAN and NPN flow in the ileum. The values obtained were -0.39 and -1.49 respectively, and were further used in the N-flow model. To prevent calculation errors with negative digestibilities for endogenous AAN, total SDAAN supply to the animal was corrected for endogenous SDAAN (eq. 2.18), which was included in the requirements (eq. 4.3).

The digestibility of SS in the small intestine was calculated according to Nocek & Tamminga (1991) (eq. 2.4). Table 2 gives a summary of the digestibility coefficients used for calculations.

The amounts of each component either digested in the small intestine or leaving the small intestine were calculated from the flow to the small intestine and the respective digestibility coefficients (eq. 2.5 to 2.22).

Large intestine

The input in the large intestine compartment consisted of undigested rumen escape feed and rumen microbial and endogenous components. For these three contributions, material fermented in the large intestine and escaped from large intestine was calculated analogous to the rumen fermentation and escape, but with appropriate passage and degradation rates (eq. 3.1a to 3.15). Also microbial synthesis and composition of microbial DM in the large intestine were assumed to be analogous to that in the rumen (eq. 3.16 to 3.28). Material digested in the large intestine was calculated as the difference between fermented and microbial production (eq. 3.29 to 3.32). The excretion of components with the faeces consisted of large intestine escape and microbial components (eq. 3.33). The large intestine N balance was calculated as the difference between fermented and microbial synthesis (eq. 3.34).

Metabolism

The metabolism compartment of the N-flow model describes the use of AAN and NPN that was digested in the small intestine (SDAAN and SDNPN) for different metabolic processes: maintenance, retention or mobilisation of body reserves and milk production.

These processes result in excretion of N with the milk, urine, faeces and skin or retention in body reserves.

Maintenance

Maintenance requirements were separated in requirements for loss of scurf-N, endogenous urinary-N and endogenous metabolic faecal-N. Requirements of SDAAN for skin and endogenous urine were related to body weight and calculated according to NRC (1985) (eq. 4.1 and 4.2).

The SDAAN requirements for the replacement of metabolic faecal N were equal to the endogenous AAN excretion in the small intestine divided by an assumed efficiency by which this endogenous AAN is synthesised from SDAAN (0.80) (eq. 4.3).

Retention and mobilisation

The requirement of SDAAN for retention of body protein or supply of SDAAN from mobilisation of body reserves was calculated from the energy balance of the animal as used in the DVE-system (Tamminga *et al.*, 1994). If the animal was in a positive energy balance, energy and AAN are deposited in body reserves. A negative energy balance resulted in mobilisation of energy and AAN. It was assumed that body reserve energy contains 10% protein. One thousand kJ NEL body reserve contains then: 1000 x 0.10 x 4.18 (kJ/NEL) / 24 (kJ/g protein) = 17.4 g body protein. Although the efficiency of SDAAN for body N varies with age (0.40 to 0.75), the average value of 0.50 from the NRC (1985) was adopted. Mobilised AAN was assumed to be converted to milk protein with an efficiency of 0.80 (Tamminga *et al.*, 1994) (eq. 4.5 to 4.11b).

Gestation

Requirements of SDAAN for growth and maintenance of maternal and foetal tissues was calculated from the nuber of days pregnant according to NRC (1985) (eq. 4.4).

Milk production

In the N-flow model, the efficiency of milk protein synthesis (YL) was dependent on the SDAAN and NEL available for milk production and milk production level (Van Straalen *et al.*, 1994a). For the establishment of the relationship a dataset of 5 feeding experiments with in total 22 treatments was used (dataset 1: Rijpkema *et al.*, 1990; Valk *et al.*, 1990; Rijpkema, unpublished data from ID-DLO, Lelystad, The Netherlands). The average and range of feed intake in dataset 1 is given in Table 4. Total intake of SDAAN and NEL was predicted and corrected for maintenance and body reserve requirements (eq. 4.12). The YL was estimated from the calculated SDAAN available for milk protein production divided by the observed milk protein production, and related to the SDAAN/NEL for milk production ratio. The relationship obtained was:

$$YL = 1.61 - 100.1 \times SDAAN/NEL \qquad (R^2 = 0.84, n=22, \text{eq. } 4.14)$$

Prediction of N-excretion

The N-flow model predicts the excretion of N with the milk, faeces, urine or skin or deposition in body protein. Faecal N-excretion is the sum of undigested escape feed N, undigested rumen microbial N, undigested not fermented endogenous N and microbial N synthesised in the large intestine. N-excretion with the urine is the sum of rumen and large intestine digested N, endogenous urinary-N, inefficient use of SDAAN for the production of milk, endogenous and body protein and SDNPN.

Sensitivity analysis

The influence of parameter values on the N-flow in the intestine and N-excretion with milk, faeces and urine was tested using a standard diet. This diet consisted of grass silage (25.0% of DM), corn silage (25.0%), sugarbeet pulp (12.5%), citruspulp (5.0%), soyabean hulls (8.5%), soyabeanmeal (7.5%), cornglutenfeed (7.5%), rapeseedmeal (5.0%), molasses (2.5%) and minerals and vitamins (1.5%). Effects were tested at a DM-intake level of 20 kg/d with a cow of 600 kg liveweight. The calculated composition of the diet in g/kg DM was as follows (with S and I fraction between brackets) OM: 910 (0.26, 0.11), CP: 186 (0.34, 0.05), LI: 28 (0.0, 0.15), SS: 163 (0.70, 0.0), NDF: 398 (0.0, 0.19), FP: 55 (1.0, 0.0) and MC: 80 (0.10, 0.15). Figure 1 shows the composition of total N and predicted flow through the animal. The parameters tested and basal, minimum and maximum parameter values are summarised in Table 5. The sensitivity of flow and excretion to parameter variation was calculated as the change in flow or excretion relative to the change in parameter value:

$$\text{sensitivity} = \frac{(f_1 - f_2) / f_b}{(p_1 - p_2) / p_b} \times 100\ \%$$

in which f_1 and f_2 are the flow or excretion values for the minimum (p_1) and maximum (p_2) parameter value, and f_b and p_b the flow or excretion for the basal parameter value.

Validation

Validation of the predicted flow of components of OM at the end of the duodenum was carried out using data from duodenal flow measurements. Data of Südekum (1989) and Tamminga (unpublished data from ID-DLO, Lelystad, The Netherlands) included flows of all components of the OM and were therefore combined (dataset 2). Duodenal flows measured by Vérité and Peyraud (unpublished data from INRA, Rennes, France) included

only OM and CP and were validated separately (dataset 3). The predicted flow of all components of the OM at the end of the ileum was validated using data from Südekum (1989). Validation of faecal excretion of OM was carried out using dataset 1 and 2 and N-balance experiments (dataset 4: Van der Honing *et al.*, unpublished data from ID-DLO, Lelystad, The Netherlands). Dataset 4 was also used to validate urinary N excretion. Milk N excretion was validated using feeding experiments (dataset 5; Veen *et al.*, unpublished data from CLO-Institute for Animal Nutrition 'De Schothorst', Lelystad, The Netherlands). Table 4 summarizes the feed intake in the experiments used.
The difference between the observed and predicted flows and excretions was calculated as the mean square prediction of error (MSPE), according to Bibby and Toutenburg (1977):

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

in which O_i and P_i is the observed and predicted values respectively, and n is the number of observations. The square root of the MSPE expressed as percentage of the observed mean is used as measure of the prediction error. The MSPE was decomposed into the error due to overall bias (intercept different from 0), the error due to deviations of the regression slope from 1 and, the error due to disturbances (unexplained variation) (Bibby and Toutenburg, 1977). Model calculations and statistical analysis were carried out using the Genstat software (Genstat 5 Committee, 1987).

Results

Sensitivity analysis

The results of the sensitivity analysis are summarised in Table 5. The AAN and NPN flow and excretion predicted by the N-flow model using the standard diet is given in Figure 1. An increase in the passage rate of particles in the rumen resulted in an increase in escape feed, endogenous and total N flow, and a decrease in microbial N flow in the duodenum. Particle passage rate did not affect escape feed N in the ileum, but for microbial, endogenous and total N flow the same tendencies as in the duodenum were observed. Flows were more sensitive to passage rates of concentrate particles than to passage rates of roughage particles. The passage rate of fluid and degradation rate of the soluble fraction hardly influenced the flow and excretion of N. Increasing the rate of degradation of feed components resulted in a lower flow of escape and endogenous CP which was almost completely compensated by a higher flow of microbial CP, due to an increase in substrate and efficiency.

Table 4. Average, minimum and maximum observed intake of DM, and predicted intake of OM, CP, LI, SS, NDF, FP, MC, NPN and AAN

	DM	OM	CP	LI	SS	NDF	FP	MC	NPN	AAN
	----- (kg/d) ----		----------				(g/d)	----------		
Dataset 1: Rijpkema *et al.* (1990 and unpublished), Valk *et al.* (1990); n=22										
Average	19.0	17.2	3312	731	2649	7670	792	1991	161	369
Minimum	17.7	15.9	2660	607	737	6782	0	1361	135	284
Maximum	19.9	18.2	426	927	4064	8979	1168	3838	239	502
Dataset 2: Tamminga *et al.* (unpublished), Südekum (1989); n=19										
Average	14.5	13.5	2651	536	3283	5318	0	1658	116	308
Minimum	7.9	7.3	1157	213	1977	3171	0	535	49	136
Maximum	19.5	17.9	4220	1325	4819	7454	0	3129	183	493
Dataset 3: Vérité *et al.* (unpublished); n=11										
Average	15.4	15.4	2430	576	3722	6690	480	1485	137	251
Minimum	14.2	12.4	1443	478	452	5465	0	791	81	145
Maximum	20.4	19.2	3016	670	5658	7697	948	3337	172	328
Dataset 4: Van der Honing (unpublished); n=29										
Average	15.5	14.1	2953	644	2761	5664	193	1868	128	344
Minimum	10.6	9.6	1801	443	394	3606	0	971	77	210
Maximum	19.4	18.0	3851	1552	5081	8026	989	3166	188	469
Dataset 5: Veen *et al.* (unpublished); n=30										
Average	20.0	18.1	3189	669	4579	7001	970	1639	155	355
Minimum	16.8	15.2	2565	524	3228	5136	584	726	124	274
Maximum	25.4	23.6	4270	900	6046	9551	1424	2263	192	492

Increasing the endogenous excretion in the small intestine resulted in a substantial increase in ileal N flow and faecal N excretion. The digestibility coefficients of escape feed, microbial and endogenous AAN and NPN had an important influence on the flow of

N in the ileum and faecal N excretion. Ileal N flows and faecal N excretion were most sensitive to variation in digestibility coefficients of escape feed and microbial AAN. An increase in the digestibility in small intestine also resulted in a lower faecal excretion of microbial N.

A higher degradation rate of undigested escape feed components in the large intestine resulted, because of the assumed constant total tract digestibility of feed CP in an increased flow of undigested escape feed CP to the large intestine, and subsequent increased microbial CP excretion with the faeces. An increase in degradation rate of endogenous and microbial components in the large intestine caused a decrease in faecal N excretion. The passage rate of concentrate particles showed a large effect on the excretion of N with the faeces and urine.

Validation

The average observed and predicted flow of components of OM through the gastrointestinal tract and N-excretion with urine and milk for the different datasets are given in Table 6. Duodenal OM flow was on average underestimated with 75 g/d for dataset 2 and 310 g/d for dataset 3. Prediction errors were mainly due to disturbances. Also duodenal CP flow was underestimated (128 g/d for dataset 2 and 36 g/d for dataset 3). The MSPE calculated for dataset 2 was due to overall bias and disturbances. For the dataset 3, the MSPE was larger and mainly due to regression and disturbance. The relationship between observed and predicted duodenal CP flows for the different datasets is shown in Figure 2a.

The predicted proportion of escape feed CP, microbial CP and endogenous CP in total duodenum CP flow were on average 40% (range 30% to 51%), 45% (range 38% to 56%) and 15% (12% to 21%) respectively. The efficiency of microbial CP synthesis was on average 0.13 g/g fermented OM, with a variation of 0.08 to 0.15 g/g. Although the average prediction of the flow of LI, SS, NDF and MC was close to the observed values in dataset 2, the MSPE was larger compared to the flow of CP and total OM. For the prediction of LI most error was due to overall bias, while for SS, NDF and MC it was due to disturbances.

The N-flow model overestimated ileal flow of both OM and CP with respectively 344 g/d and 10 g/d (dataset 2, Table 6). For OM this error was due to overall bias and disturbance, while for CP the regression and disturbance were the main components of the MSPE. The relationship between N-flow model predictions and observed values of ileal CP flow is given in Figure 2b. As for duodenal flows, prediction errors of ileal flow of LI, SS and NDF were larger than for ileal CP flow. The prediction error of LI and NDF were mainly due to overall bias, while for SS the disturbance was the main contribution to the prediction error. The predicted MC was close to the observed values, with most error due to disturbances. Of the predicted ileal CP flow 17% originated from escape feed CP (range 10% to 26%), 17% from microbial CP (12 to 20%) and 66% from

Table 5. Sensitivity of the CP flow in the beginning of the small intestine, large intestine and faecal CP-excretion to variation in parameter values

Parameter	Unit	Parameter Basis	Variation Min.	Max.	Sensitivity of N flow or excretion (%) — Small intestine — Esc.	Mic.	End.	Tot.	Large intestine — Esc.	Mic.	End.	Tot.	Faeces — Esc.	Mic.	End.	Tot.
Rumen																
Passage rate roughage particles	(%/h)	4	2	6	11	-3	4	4	0	-2	4	2	0	0	4	2
Passage rate concentrate particles	(%/h)	6	3	9	30	-6	8	10	0	-6	7	4	0	-1	8	4
Passage rate fluids	(%/h)	10	5	15	3	-2	1	1	0	-2	1	1	0	0	1	1
Degradation rate feed components	(%/h)	0.5x[1]	1.0x	1.5x	-41	38	-20	-3	0	39	-20	-7	0	25	-20	-4
Degradation rate S-fractions	(%/h)	300	150	450	0	0	0	0	0	0	0	0	0	0	0	0
Small intestine																
Endogenous CP excretion	(g/kg OM)	60	30	90	0	0	100	15	0	0	100	67	0	6	100	58
Digestibility escape AAN	(%)	86	78	95	-	-	-	-	-369	0	0	-61	-366	-5	0	-59
Digestibility escape NPN	(%)	75	67	82	-	-	-	-	-127	0	0	-21	-136	-1	0	-22
Digestibility endogenous AAN	(%)	-40	-80	0	-	-	-	-	0	0	-36	-24	0	-2	-36	-21
Digestibility endogenous NPN	(%)	-150	-200	-100	-	-	-	-	0	0	-64	-43	0	-4	-64	-37
Digestibility microbial AAN	(%)	85	75	95	-	-	-	-	0	-394	0	-67	0	-207	0	-58
Digestibility microbial NPN	(%)	80	70	90	-	-	-	-	0	-123	0	-21	0	-65	0	-18
Large intestine																
Degradation rate escape feed	(%/h)	0.5x[1]	1.0x	1.5x	-	-	-	-	20	0	0	3	0	15	0	4
Degradation rate endogenous	(%/h)	5	2.5	7.5	-	-	-	-	-	-	-	-	0	21	-30	-11
Degradation rate microbes	(%/h)	5	2.5	7.5	-	-	-	-	-	-	-	-	0	-14	0	-4
Passage rate roughage particles	(%/h)	14	10	18	-	-	-	-	-8	0	0	-1	0	-5	0	-1
Passage rate concentrate particles	(%/h)	14	12	16	-	-	-	-	-11	0	0	-2	0	-16	26	11

[1] factor by which rate of degradation was multiplied

Figure 2. Relationships between predicted and observed flow of CP in the duodenum (a), ileum (b) and excretion of N with the faeces (c), urine (d) and milk (e). Data from: Tamminga *et al.* (unpublished) (△); Südekum (1989) (□); Vérité *et al.* (unpublished) (○); Van der Honing *et al.* (unpublished) (◇) and Veen *et al.* (unpublished) (▽)

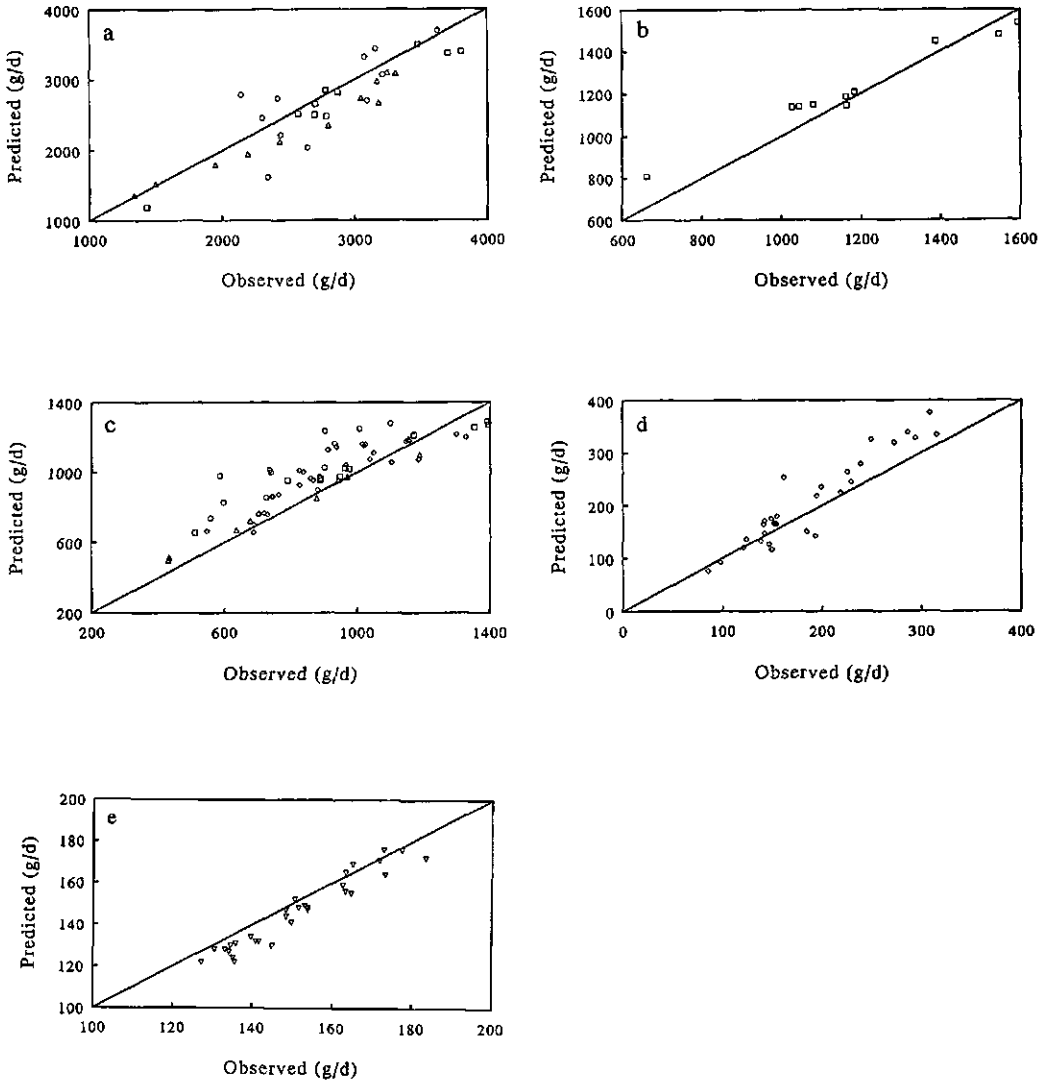


Table 6. Average predicted and observed flow in the beginning of the small intestine, large intestine and faecal excretion of OM, CP, LI, SS, NDF and MC, and excretion of N with urine and faeces, prediction error and contributions to the MSPE

Measurement	Item	n	--- Average (g/d) --- Observed	Predicted	Prediction Error (%)	---------- MSPE due to (%) ---------- Bias	Regression	Disturbance
Dataset 2: Tamminga (unpublished) and Südekum (1989)								
Duodenal flow	OM	19	8015	7940	6.3	2.2	0.8	96.9
	CP	19	2831	2703	8.3	29.8	6.7	63.5
	LI	19	631	786	28.5	75.5	2.8	21.7
	SS	19	341	364	39.4	2.8	1.5	95.7
	NDF	19	1921	1995	25.8	2.2	0.0	97.8
	MC	19	2291	2093	18.0	23.1	7.3	69.6
Ileal flow	OM	10	5087	5431	8.9	58.5	5.4	36.2
	CP	10	1282	1292	5.8	1.8	49.9	48.3
	LI	10	97	115	23.1	62.4	3.7	33.9
	SS	10	89	77	58.2	5.1	0.0	94.8
	NDF	10	1756	2141	25.9	71.6	14.2	14.2
	MC	10	1863	1806	7.7	15.3	15.0	69.7
Faecal excretion	OM	19	3684	3863	8.7	31.5	3.2	65.3
	CP	19	942	964	8.0	8.2	32.7	59.1
	LI	19	137	133	31.3	0.7	16.0	83.4
	SS	19	27	62	172.6	53.5	30.5	16.0
	NDF	19	1497	1624	30.7	7.6	0.8	91.5
	MC	19	1080	1080	31.4	0.0	30.2	69.8

Table 6. Continued.

Dataset 3: Vérité and Peyraud (unpublished)								
Duodenal flow	OM	11	9464	9154	5.9	30.3	13.1	56.6
	CP	11	2768	2732	14.6	0.8	41.2	58.0
Faecal excretion	OM	11	4582	4831	12.6	18.5	32.9	48.6
	CP	11	799	1033	30.9	90.1	0.5	9.4
Dataset 4: Van der Honing (unpublished)								
Faecal excretion	N	29	148	159	12.0	38.3	0.1	61.6
Urine excretion	N	29	188	207	19.6	28.2	35.2	36.6
Dataset 5: Veen *et al.* (unpublished)								
Milk excretion	N	30	151	146	4.8	52.2	11.5	36.3

endogenous CP (60 to 74%).

Faecal OM excretion was on average overestimated by 179 g/d for dataset 2 and 249 g/d for dataset 3. In both datasets most error was due to disturbances. Also the excretion of CP was on average overestimated (22 g/d for dataset 2, 234 g/d for dataset 3 and 68 g/d for dataset 4. Data from dataset 2 and 4 showed a better fit than from dataset 3, in which most MSPE was due to overall bias (Figure 2c, Table 6). The predicted faecal CP originated from: 17% escape feed CP (range 11% to 26%), 14% rumen microbial CP (9% to 16%), 56% endogenous CP (48% to 65%) and 14% microbial CP, synthesized in the large intestine (11% to 19%). Although absolute difference between faecal excretion of LI and MC were low, relationships were poor: the MSPE's were mainly due to disturbances. Faecal SS and NDF flows were overestimated. For SS the MSPE was mainly made up from overall bias, while for NDF the error was almost only due to disturbances.

The N-flow model overestimated the excretion of N with the urine with on average 22 g/d (dataset 4, Table 6; Figure 2d). All sources of error contributed to the MSPE. Contributions to urinary N in dataset 4 were: 9% endogenous urinary-N (range 4% to 21%), 42% N-loss in the rumen (-60% to 83%) and 17% N-loss in the large intestine (7% to 37%), 6% N lost with urine due to synthesis of gastrointestinal endogenous-AAN (2% to 12%), 28% due to milk-N production (0% to 84%) and -3% digested NPN (-29% to 27%). The latter value is the difference between NPN absorption in the small intestine (feed and microbial) and endogenous NPN excretion in the gastro-intestinal tract.

Of the SDAAN in dataset 5 on average 6% was used for endogenous urinary N (4% to 7%), 1% for skin (1% to 2%), 1% for body reserves (-2 to 10%), 24% for gastrointestinal endogenous AAN (22% to 26%) and 68% for milk production (59% to 74%).

Milk-N excretion was underestimated in dataset 5 (Table 6). The relationship between predicted and observed values was close and is given in Figure 2e. The efficiency of milk protein production (g milk protein/g digested amino acids available for milk production) in dataset 5 was on average 74%, with a variation of 58% to 84%.

Average output of N with the skin, body, faeces, urine and milk as percentage of N intake, using all datasets, were respectively: 0 (range 1% to 0%), 0% (-6% to 5%), 37% (21% to 55%), 38% (14% to 70%) and 24% (0 to 34%).

Discussion

Feedstuff composition

Part of the parameter values in the N-flow model were based on assumptions. Because of lack of data, the degradation characteristics of MC in the feed were assumed to be constant for all feedstuffs. However, the size and composition of the MC may differ

between feedstuffs. The MC fraction varied between feedstuffs from 0 to 350 g/kg DM. Feedstuffs with a high MC content were for example citruspulp and vinasses. This fraction may include among other things pectines, fructosanes and galactosides. Also analytical errors of other OM components accumulate in MC. For instance the N in NDF will be accounted for in the N content and NDF content. For some feedstuffs the NDF content was adjusted to prevent MC from being negative. Of the total OM intake in dataset 2, on average 12% was MC.

Rumen compartment and duodenal flow

Although the results of the sensitivity analysis depend on the basal diet used, it showed that varying some parameter values had only a minor influence on the predictions of the duodenal CP flow. Ruminal fractional passage rate of fluid and degradation rate of the S-fraction only showed limited effects on the fermentability of the S-fractions, because of the relative high assumed degradation rates of the S-fractions compared to passage rates of fluid. On average 96% of the S-fractions were predicted to be degraded in the rumen. The fractional passage rates of concentrates and endogenous CP excretion showed a larger effect on duodenal CP flow than the passage rate of roughage particles. This can partly be explained by the fact that roughages usually have a higher NDF content than concentrates, for which a different approach was followed in the N-flow model than for the other components of the OM.

Total CP flow in the duodenum was underestimated by the N-flow model. The contribution of microbial CP to total duodenal CP predicted by the N-flow model (on average 45%) was lower than observed in literature for a large range of different feed intakes and diets (from 56 to 67%; Robinson and Sniffen, 1985; Robinson *et al.*, 1985; Van Vuuren *et al.*, 1993). This indicates underestimation of the microbial CP flow, and thus overestimation of the escape CP flow. However, this conclusion should be treated with care because of the low accuracy by which microbial CP flow can be measured using cows with a T-type cannula and DAPA as a marker (Broderick and Merchen, 1992). The sensitivity analysis showed that the ratio between microbial and escape feed CP flow could be increased by increasing the fractional degradation rate of the feed, and thus indicates that the degradation rates measured by nylon bag incubations in the rumen might be an underestimation of *in vivo* values. The increase in microbial CP flow with increasing degradation rate is due to more fermentable OM and higher efficiency of microbial DM synthesis, and remains evident along the total gastrointestinal tract. The efficiency of microbial DM synthesis was adapted from the CNCPS-system (Russell *et al.*, 1992). With this system a close relationship was observed between predicted and measured bacterial CP flow in the duodenum (Russell *et al.*, 1992). Direct comparison of the CNCPS with our model remains difficult because degradation characteristics in the CNCPS were described differently and obtained from both *in vitro* and *in situ* studies. Compared to various protein evaluation systems (NRC, 1985; Vérité *et al.*, 1987;

Tamminga *et al.*, 1994), the efficiency of microbial CP synthesis predicted by the N-flow model was 10 to 15% lower.

Endogenous OM arriving in the small intestine consists of unfermented saliva, sloughed off epithelium cells from stomach and beginning of the small intestine and pancreatic and biliary excretions. The average endogenous contribution in duodenal CP was in line with values reported by Kaufmann and Hagemeister (1976) for dairy cows (15%) and by Siddons *et al.* (1982) for sheep (20%) but was higher than the value calculated by regression analysis (6%) by Vérité *et al.* (1987), who used sheep, steer and dairy cow data.

The N-flow model showed a better prediction of duodenal CP flow for Dataset 2 than for Dataset 3. This could be due to the type of the canula in the duodenum: in Dataset 2 re-entrant canulas were used, while in Dataset 3 T-shape cannula's were used. The latter measurements are less accurate because of the inevitable application of flow markers (Owens and Hanson, 1992).

As discussed by Tamminga *et al.* (1989) duodenal NDF flow could not be estimated by the combination of passage rates (measured with markers) and degradation characteristics (measured with nylon bag incubations). Calculations with the N-flow model supported this conclusion. When passage rate of NDF (measured with Cr-mordanted NDF) was assumed to be half of that of total OM (Tamminga *et al.*, 1989; Robinson and Kennelly, 1991; Van Vuuren *et al.*, 1993) and degradation characteristics measured with nylon bags were used, the flow of NDF in the duodenum was heavily overestimated (in dataset 2 on average 665 g/d, MSPE = 47%). This implies that the mentioned techniques overestimate the passage rate or I-fraction of NDF or underestimate the degradation rate. It could also be concluded that the assumption that the rumen behaves like a total mixing compartment is not valid. The extent of NDF fermentation depends on a number of factors, which are not all taken into account with the different methods to estimate NDF fermentation. During nylon bag incubation feedstuffs are not subjected to rumination. To mimic the rumination process, feedstuffs were ground or chopped before incubation. Even then the I-fraction may be higher and degradation rate lower compared to *in vivo*. Particle size and functional specific gravity, assumed to be the major factors controlling NDF clearance from the rumen (Tamminga, 1993), were not taken into account in the N-flow model. The functional specific gravity of particles increases during digestion and rumination as particles become smaller and gass spaces, as result of microbial fermentation, are removed. Because of lack of fermentation, indigestible feed particles may have a higher passage rate than particles which are still being digested (Tamminga *et al.*, 1989). However, the prediction of NDF flow in the N-flow model was not improved when passages rate of NDF was related to the I-fraction of the NDF. Tamminga (1993) calculated that the proportion of the D-fraction of NDF fermented in the rumen varied with level of feeding between 0.70 g/g at 6 kg DM intake to 0.85 at 24 kg DM intake. In contrary to what was expected, relating the proportion of the NDF fermented in the rumen to DM intake showed a larger MSPE than using a constant proportion of the D-

fraction of NDF fermented in the rumen (0.80). Therefore this constant factor was subsequently used in the final version of the N-flow model and thus rate of passage was directly related to the rate of degradation ($kp1_{NDF}$ = 0.25 x $kd1_{NDF}$; eq. 0.4a and 0.4b).

The duodenal LI flow was overestimated by the N-flow model, with a relatively high prediction error mainly due to bias. The prediction error could be caused by the assumed lack of rumen fermentation of LI in the N-flow model. Jenkins (1993) calculated a disappearance of feed LI in the rumen of 0.08 and Doreau and Ferlay (1994) obtained a disappearance of feed fatty acids in the rumen of 0.20. The latter also calculated a microbial fatty acid contribution to duodenal LI flow of 9 g/kg DM intake, which seems to correspond with total LI of microbial origin predicted by the N-flow model (on average 16 g/kg DM intake). However, only a very limited number of data for individual feedstuffs are available (Perrier *et al.*, 1992) which could be used for a possible better description of LI digestion in the N-flow model.

The SS flows showed a high MSPE with most error unexplained (Table 6). This error is possibly due to difficulties to measure SS fermentation with the nylon bag technique. In the N-flow model no correction for pelleting of feedstuffs on SS degradation characteristics as suggested by Nocek and Tamminga (1991), was carried out.

The values assumed for the I-fraction of feed MC (0.15) and excretion of endogenous MC in the duodenum (0.18 g/kg OM passing) were iteratively chosen in order to minimize the difference between observed and predicted flow of MC. At duodenal level, part of the MC flow may originate from NDF and SS converted into MC (α-gluconpolymers) in microbes. A further improvement of the flow of MC can possibly be achieved after a more detailed study of measured MC flow. Although it could be of importance for the predicted N-flow by the N-flow model, this was considered to be beyond the scope of this paper.

Small intestine compartment and ileal flow

Variation of intestinal digestibility of escape feed, microbial and endogenous AAN and NPN showed a large effect on ileal CP flow. The calculation of the digestibility of escape feed N was based on the assumption that each feedstuff contained a constant indigestible fraction, irrespective of the escape fraction (Hvelplund *et al.*, 1992; Van Straalen *et al.*, 1993). The digestibility coefficient was dependent on the extent of fermentation in the rumen and large intestine, and thus on degradation characteristics and fractional passage rates in the rumen and large intestine. The average values predicted in dataset 2 for escape feed AAN and NPN were respectively 0.86 and 0.71. The assumption that the content of AAN in total N in escape feed was similar to that in the feedstuff is debatable. For most feedstuffs it was observed that AAN is less degradable than NPN, but differences do exist between feedstuffs (Van Straalen *et al.*, 1994b). The lower AAN content in escape protein assumed in the N-flow model compared to observed values may have only a minor influence on the SDAAN supply to the animal, because it is partly compensated

by the fact that a higher AAN digestibility is calculated compared to NPN or total N.

Because dataset 2 was used for developing the digestibility coefficients for endogenous CP, ileal CP flow could not be validated against these data. An indirect validation of the assumed values for the digestibility of escape feed and microbial AAN and NPN was carried out by estimating digestibility coefficients using multiple regression analysis. Dataset 2 and assumptions, as used for the estimation of digestibility coefficient of endogenous AAN and NPN, were used (see Model description). In the multiple regression analysis the 'observed' flow of AAN and NPN flow at the end of the ileum (IL) was related to the predicted flow of escape feed (RE), microbial (RM) and endogenous (RG) AAN and NPN at the end of the duodenum:

$$ILAAN = 0.07 \times REAAN + 0.26 \times RMAAN + 1.16 \times RGAAN \quad (R^2=0.96, n=19)$$
$$ILNPN = 0.23 \times RENPN + 0.98 \times RMNPN + 1.35 \times RGNPN \quad (R^2=0.96, n=19)$$

The regression coefficients are an estimate of one minus the digestibility coefficient of each component, thus the estimated digestibility of AAN and NPN were 0.93 and 0.77 respectively. This indicated that the N-flow model underestimates digestion of escape feed AAN, which was on average 0.85. The digestibility of microbial AAN and NPN were fixed at 0.85 and 0.80, respectively. Using the multiple regression approach, lower values were obtained, especially for microbial NPN (0.77 and 0.02 respectively). An explanation for the higher digestibility of escape feed N and lower value for microbial N is the underestimation of microbial N flow in total duodenal N as discussed earlier. The digestibility of endogenous AAN and NPN obtained from the multiple regression analysis (-0.16 and -0.35 respectively) were higher than the values used in the N-flow model. They were still considerably lower than values reported for sheep using the ^{15}N method (0.50; Voigt *et al.*, 1993) or for pigs using the digesta exchange ^{15}N method (0.80; Krawilitzki *et al.*, 1990). From comparison of amino acid profiles, Van Bruchem *et al.* (1985) calculated that with sheep 73% of ileal N flow was of endogenous origin. This value is close to the one predicted by the N-flow model (66%).

The overestimation of ileal OM flow was caused by overestimation of the NDF flow. In the N-flow model no digestion of NDF in the small intestine was assumed. Südekum (1989) observed digestibility coefficients between -0.10 to 0.14, which was explained by microbial fermentation in the small intestine or reflux of caecal digesta into the end of the small intestine.

The LI flow in the ileum was overestimated by the N-flow model. Besides escape feed LI also microbial LI contributed to this flow (on average 1/3 of total LI flow). Both sources were assumed to be digested for 0.85. If the overestimation of LI flow at the duodenal level is accounted for, this value showed to be a good estimate. In the N-flow model no contribution of endogenous LI was assumed. Including this source of LI in the N-flow model would imply a higher digestibility of escape feed LI and microbial LI. However, the LI flow is not important for the prediction of N flow.

The flow of SS in the ileum was underestimated, with an error which was mainly unexplained. This indicates that the formula proposed by Nocek and Tamminga (1991) does not seem correct for this database. The N-flow model predicted that for all datasets on average 80% of total intestinal SS digestion took place in the small intestine, which is in contrast to data presented by Sauvant *et al.* (1994), who concluded that the small intestine accounted only for 40% of total intestinal SS digestion.

Given the digestibility of escape feed MC, the endogenous MC flow in the ileum was calculated by the N-flow model to be on average 80% of the total MC flow, which was about one third of the total OM ileal flow (Table 6). These figures emphasise the importance of this endogenous contribution, of which no information is available in literature.

Large intestine compartment and faecal excretion

Hardly any information was available to obtain parameter values that describe the fermentation in the large intestine, while some of these assumptions had a pronounced influence on the N-excretion with the faeces. The passage rate in the large intestine was calculated from TMRT and kp1, and the transit time in the small and large intestine. The time spend in the small and large intestine was based on their volumes (O'Connor *et al.*, 1984). In this approach transit time in omasum and abomasum was not taken into account. Including these parts of the gastrointestinal tract minus rumen and reticulum results in a lower contribution of the large intestine to the total volume (0.25 in stead of 0.40), and would increase the calculated passage rate in the large intestine (0.22/h in stead of 0.14/h at 20 kg DM intake). However, increasing the passage rate of feed particles in the large intestine did not have any influence on escape feed N excretion with the faeces, because of the assumed constant indigestible N fraction per feedstuff. Increasing the passage rate would shift the digestion of escape feed N from the large intestine to the small intestine, as shown by the decrease in escape feed N flow at the end of the ileum. As a result of a higher passage rate less material would be fermented in the large intestine and the microbial N excretion with the faeces will decrease. The opposite effect was observed when increasing the degradation rate of undigested escape feed components. The increase of faecal N excretion with increasing passage rate of concentrates is due to the fact that this passage rate was also used for endogenous material.

On average 15% of the undigested escape feed N that entered the large intestine was fermented. With the mobile nylon bag method values between -37 to 68% were observed (Hvelplund, 1985; Voigt *et al.*, 1985). However, the absolute amount of escape feed N digested in the large intestine remains limited compared to that in the small intestine.

At high intake levels the N-flow model tended to underestimate faecal OM and N excretion (Figure 2c). The proportion of microbial N (from rumen and large intestine) in total faecal N (28%) was considerably lower than values reported in literature (65%;

Mason, 1969; Robinson and Sniffen, 1985; Robinson *et al.*, 1987). Many factors can contribute to this discrepancy; underestimation of microbial CP synthesis in the rumen and large intestine and overestimation of the digestibility in the small intestine. Microbial CP synthesis in the large intestine was assumed to be analogous to that in the rumen. Although differences between rumen and hindgut in fermentation pattern and efficiency have been reported (Demeyer and De Graeve, 1991), quantitative information on large intestinal fermentation is limited. Also fractional passage and degradation rates of endogenous components had a marked influence on faecal CP excretion. The excretion of endogenous CP was on average 136 g/kg faecal OM, which is twice as high as the value that was calculated by regression analysis (60 g/kg OM) by Vérité *et al.* (1987). A higher degradation rate in combination with a lower passage rate of endogenous CP in the large intestine would result in a lower endogenous and higher microbial CP excretion with the faeces. However, the large intestine N balance was generally positive, indicating that fermentation of OM and not of CP was limiting microbial synthesis.

The overestimation of excretion of OM with the faeces was mainly due to NDF. Although this could be the result of the overestimated ileal NDF flow, it also suggests an underestimation of the fermentation in the large intestine. The predicted fermentation of NDF in the large intestine was in dataset 2 on average 6% of total digested NDF, which is lower than the value adopted by Robinson *et al.* (1987) of 15% of digested NDF. This would imply a too low assumed degradation rate and too high passage rate of NDF in the large intestine. Also the indigestible fraction after 14 days rumen incubation could be an overestimation of total tract indigestibility.

Metabolism compartment and urinary and milk N-excretion

The overestimation of N excretion with the urine was more pronounced at high intake levels (Figure 2e). Although this agreed with the underestimation of N excretions with the faeces at high intake levels, for individual treatments this was not always the case.

The contribution of different N sources to urinary N excretion varied largely between observations. Urinary N losses can be regarded as inevitable (endogenous urinary-N and skin-N), to a lesser extend avoidable (endogenous metabolic faecal-N, digested NPN) and more or less avoidable (inefficient milk-N synthesis, rumen and large intestine N balance). Using all datasets these proportions were respectively 31%, 45% and 23% of urinary N, without correction for NPN excreted via the intestines. Endogenous metabolic faecal-N was related to the flow of OM in the gastrointestinal tract, and can possibly be reduced by feeding high digestible diets. Digested NPN originated equally from microbial and feed source. A higher reclycing of NPN in the rumen might reduce this excretion. The loss of N in the rumen and large intestine and N-loss due to inefficient milk N-synthesis is the result an imbalance between energy and CP fermentation or metabolisation and can be largely influenced by balancing dietary composition.

About 30% of total SDAAN was used for maintenance requirements, and 70% for

production purposes, which is close to the proportions as observed for protein evaluation systems in which maintenance requirements is related to feed intake (Van Straalen *et al.*, 1994a). The efficiency of milk N production depended on the available SDAAN and NEL for lactation. When the supply of SDAAN compared to NEL is limiting for milk production the efficiency increases. This probably caused the good relationship between predicted and observed milk N excretion. The average efficiency observed for dataset 5 was higher than used in most protein evaluation systems (Van Straalen *et al.*, 1994a). The good relationship between predicted and observed milk-N excretion was due to the variable efficiency of milk protein production. The underestimation at low production levels could be prevented by including the production level (kg milk/d) into the regression equation as was done for the DVE-system (Tamminga *et al.*, 1994), but this factor did not significantly contribute to the variance accounted for in dataset 1.

Conclusions

The detailed description of N-fluxes in the N-flow model could only be achieved when fermentation, digestion and metabolism of total OM was taken into account. For the MC, degradation characteristics in rumen and large intestine, digestibility coefficients in the small intestine and endogenous contribution were mainly based on assumptions. Because a large part of the OM flow consisted of MC, these assumptions need further research.

The N-flow model underestimated duodenal microbial N flow, probably due to a too low fermentation of OM or efficiency of microbial CP synthesis. The NDF fermentation in the rumen could not be described regarding the rumen as a total mixing compartment. Incorporation of particle size and functional specific gravity in the N-flow model needs further study. Duodenal CP flow was hardly influenced by passage rates or degradation rate of the soluble fractions.

The prediction of ileal N flow (and faecal excretion) was very sensitive to the digestibility coefficients of escape feed, microbial and endogenous N in the small intestine. Negative coefficients for endogenous AAN and NPN were necessary to prevent overestimation of ileal N flow. Because the digestion of endogenous components could not be separated from escape feed and microbial components, further research is needed in which those three sources are separated.

Faecal excretion of N was on average overestimated. Possibly due to inadequate assumptions in large intestine fermentation, the CP of microbial origin was underestimated and endogenous contribution overestimated. More information on large intestine fermentation is needed. Urinary N excretion was on average overestimated. N loss in the rumen showed to be the main, but also the most variable source of urinary N. Milk N excretion was predicted accurately because of the variable efficiency of milk protein production. Because the N-flow model gives detailed information on the source of excretions with faeces, urine and milk, it can be used to further study the possibilities of

feeding management to reduce N loss in dairy cows.

Acknowledgements

The authors are grateful to Y.S. Rijpkema, H. Valk and Y. van der Honing (ID-DLO, Lelystad), W.A.G. Veen (CLO-Institute for Animal Nutrition De Schothorst, Lelystad), K.-H. Südekum (Institut für Tierernährung und Futtermittelkunde, Christian-Albrechts Universität, Kiel, Germany) and R. Vérité and J.-L. Peyraud (INRA, Rennes, France) for the provision of data that were used for the development and validation of the N-flow model. This study was partially financed by the Fund for Manure and Ammonia Research (FOMA).

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APPENDIX: DESCRIPTION OF INPUT AND STATEMENTS IN N-FLOW MODEL

Model input and parameters

Feedstuff composition and degradation characteristics

- Components of OM (FEi) with i = CP, LI, SS, NDF, FP, MC; and N, AAN and NPN (in g/kg DM)
- Fractions for each component i: soluble (S_i), indigestible:(I_i) and potentially degradable (D_i, in g/g i)
- Fractional degradation rates in the rumen of S_i ($kd1s_i$, in %/h)
- Fractional degradation rates in the rumen of D_i ($kd1_i$, in %/h)
- Energy content (NEL, in kJ/kg DM)
- Amino acid N in total N (%AAN, in g AAN/g N)

Composition and degradation rates of microbial and endogenous components

- Composition of rumen non structural microbes ($RMNSC_i$, in g/kg DM)
- Composition of rumen structural microbes ($RMSC_i$, in g/kg DM)
- Composition of large intestine microbes (LM_i, in g/kg DM)
- Composition of endogenous OM (RG_i, in g/g OM passing in the duodenum)
- Degradation rate of microbial components in the large intestine ($kd3m_i$, in %/h)
- Degradation rate of endogenous components in the large intestine ($kd3g_i$, in %/h)
- Indigestible fraction of microbes components (IM_i, in g/g)
- Indigestible fraction of endogenous components (IG_i, in g/g)

Animal data

- DM-intake (KGDM, in kg/d)
- Proportion roughage in the diet (%R, in g/g)
- Live weight (LW, in kg)
- Days in gestation (GDAY, in day)
- Milk production (KGM, in kg/d), fat content (%FAT, in %) and protein content (%PROT, in %)

Fractional degradation rates in the large intestine ($kd3_i$)

$kd3_i = 0.5 * kd1_i$ 0.1

Fractional passage rates in the rumen of fluid (kp1f), *concentrates* ($kp1c_i$) *and roughages* ($kp1r_i$)

$kp1f = (-3.40+1.22*KGDM-0.03*KGDM^2+5.93 *\%R)/100$ 0.2

$kp1r_i = (1.74+0.15*KGDM)/100$ 0.3a

$kp1c_i = (10.08 - 0.96*KGDM + 0.04*KGDM^2)/100$ 0.3b

$kp1c_{NDF} = kd1_{NDF}*0.25$ 0.4a

$kp1r_{NDF} = kd1_{NDF}*0.25$ 0.4b

Total tract mean retention time of concentrates ($TMRTC_i$) *and roughages* ($TMRTR_i$)

$TMRTC_i = 57.65 - 1.11*KGDM$ 0.5a

$TMRTR_i = 71.84 - 1.49*KGDM$ 0.5b

Fractional passage rates in the large intestine of concentrates ($kp3c_i$) *and roughages* ($kp3r_i$)

$kp3r_i = 1 / ((TMRTR_i - 1/kp1r_i)*0.4)$ 0.6a

$kp3c_i = 1 / ((TMRTC_i - 1/kp1c_i)*0.4)$ 0.6b

$kp3r_{NDF} = kp3r_{OM}$ 0.7a

$kp3c_{NDF} = kp3c_{OM}$ 0.7b

Model calculations: rumen compartment

Rumen fermented D-fraction components (RFDi)

- For concentrate ingredients:

$RFDi = FEi * (1-I_i-S_i) * kd1_i/(kd1_i+kp1c_i)$ 1.1a

- For roughages:

$RFDi = FEi * (1-I_i-S_i) * kd1_i/(kd1_i+kp1r_i)$ 1.1b

Rumen fermented S-fraction components (RFSi)

$RFSi = FEi * S_i * kd1s_i/(kd1s_i+kp1f)$ 1.2

Rumen fermented components (RFi)

$RFi = RFSi + RFDi$ 1.3

$RFOM = RFCP + RFLI + RFSS + RFNDF + RFFP + RFMC$ 1.4

Rumen escape feed components (REi)

$REi = FEi - RFi$ 1.5

$REOM = RECP + RELI + RESS + RENDF + REFP + REMC$ 1.6

Rumen microbial efficiency on fermented D-fraction components (YMDi)

$YMDNDF = 1 / (0.15/kd1_{NDF} + 1/0.33)$ 1.7

$YMDSS = 1 / (0.05/kd1_{SS} + 1/0.33)$ 1.8

$YMDMC = 1 / (0.05/kd1_{MC} + 1/0.33)$ 1.9

$YMDCP = YMDSS/2$ 1.10

$YMDFP = YMDSS/2$ 1.11

$YMDLI = 0$ 1.12

$YMSN = YMSCP$ 1.13

$YMSAAN = YMSCP$ 1.14

$YMSNPN = YMSCP$ 1.15

Rumen microbial efficiency on fermented S-fraction components (YMSi)

$YMSi = 1 / (0.05/kd1s_i + 1/0.33)$ 1.16

YMSCP = YMSSS/2 1.17
YMSFP = YMSSS/2 1.18
YMSLI = 0 1.19
YMSN = YMSCP 1.20
YMSAAN = YMSCP 1.21
YMSNPN = YMSCP 1.22

Rumen microbial components synthesis on fermented non structural carbohydrates (RMNSCi)
RMNSCi = (RFSSS * YMSSS + RFDSS * YMDSS + RFSFP * YMSFP + RFDFP * YMDFP + RFSMC * YMSMC + RFDMC * YMDMC) * $RMNSC_i$ 1.23

Rumen microbial components synthesis on fermented structural carbohydrates (RMSCi)
RMSCi = (RFSNDF * YMSNDF + RFDNDF * YMDNDF) * $RMSC_i$ 1.24

Rumen microbial components synthesis on fermented crude protein (RMCPi)
RMCPi = (RFSCP * YMSCP + RFDCP * YMDCP) * $RMSC_i$ 1.25

Rumen microbial components synthesis (RMi)
RMi = RMNSCi + RMSCi + RMCPi 1.26
YMi = RMi/RFOM 1.27

Rumen digested components (RDi)
RDi = RFi - RMi 1.28
RDOM = RDCP+ RDLI+ RDSS+ RDNDF+ RDFP+ RDMC 1.29

Rumen output components (ROi) *and endogenous components flow to the small intestine* (RGi)
ROOM = REOM + RMOM 1.30
RGi = RG_i * ROOM 1.31
RGN = RGAAN + RGNPN 1.32
RGCP = RGN * 6.25 1.33
RGOM = RGCP+ RGLI+ RGSS+ RGNDF+ RGFP + RGMC 1.34
ROi = REi + RMi + RGi 1.35
ROOM = ROCP+ ROLI+ ROSS+ RONDF+ ROFP + ROMC 1.36

Rumen Nitrogen balance (RNB)
RNB = RFN - RMN 1.37

Small intestine compartment

Small intestine digestibility coefficients (SDE_i)
- For concentrate ingredients:
SDE_i = $(RE_i - [I_i/(kp3c_i/(kd3_i+kp3c_i))])/RE_i$ 2.1a

- For roughages:

$SDE_i = (RE_i - [I_i/(kp3r_i/(kd3_i + kp3r_i))])/RE_i$ 2.1b

$SDE_{AAN} = 0.18 + 0.82 * SDE_{CP}$ 2.2

$SDE_{NPN} = (SDE_{CP} - \%AAN*SDE_{AAN})/(1-\%AAN)$ 2.3

$SDE_{SS} = (-0.728*RE_{SS}+0.879)$ 2.4

Small intestine digested rumen escape components (SDEi)

SDEi = REi * SDE_i 2.5

SDEN = SDEAAN + SDENPN 2.6

SDECP = SDEN * 6.25 2.7

SDEOM = SDECP+SDELI+SDESS+SDENDF+SDEFP+SDEMC 2.8

Small intestine digested rumen microbial components (SDMi)

SDMi = RMi * SDM_i 2.9

SDMN = SDMAAN + SDMNPN 2.10

SDMCP = SDMN * 6.25 2.11

SDMOM = SDMCP+SDMLI+SDMSS+SDMNDF+SDMFP+SDMMC 2.12

Small intestine digested endogenous components (SGEi)

SDGi = RGi * SDG_i 2.13

SDGN = SDGAAN + SDGNPN 2.14

SDGCP = SDGN * 6.25 2.15

SDGOM = SDGCP+SDGLI+SDGSS+SDGNDF+SDGFP+SDGMC 2.16

Small intestine digested components (SDi)

SDi = SDEi + SDMi + SDGi 2.17

SDAAN = SDEAAN + SDMAAN 2.18

Small intestine output rumen escape components (SOEi)

SOEi = REi - SDEi 2.19

Small intestine output rumen microbial components (SOMi)

SOMi = RMi - SDMi 2.20

Small intestine output endogenous components (SOGi)

SOGi = RGi - SDGi 2.21

Small intestine output components (SOEi)

SOi = SOEi + SOMi + SOGi 2.22

Large intestine compartment

Large intestine fermented rumen escape components (LFEi)

- For concentrate ingredients:

LFEi = SOEi * $kd3_i$ / ($kd3_i$+$kp3c_i$) 3.1a

- For roughages

LFEi = SOEi * $kd3_i$ / ($kd3_i$+$kp3r_i$) 3.1b

LFEOM = LFECP+LFELI+LFESS+LFENDF+LFEFP+LFEMC 3.2

Large intestine fermented rumen microbial components (LFMi)

LFMi = SOMi * $kd3m_i$ / ($kd3m_i$+$kp3c_i$) 3.3

LFMN = LFMAAN + LFMNPN 3.4

LFMCP = LFMN * 6.25 3.5

LFMOM = LFMCP+LFMLI+LFMSS+LFMNDF+LFMFP+LFMMC 3.6

Large intestine fermented endogenous components (LFGi)

LFGi = SOGi * $kd3g_i$ / ($kd3g_i$+$kp3c_i$) 3.7

LFGN = LFGAAN + LFGNPN 3.8

LFGCP = LFGN * 6.25 3.9

LFGOM = LFGCP+LFGLI+LFGSS+LFGNDF+LFGFP+LFGMC 3.10

Large intestine fermented components (LFi)

LFi = LFEi + LFMi + LFGi 3.11

Large intestine escape of rumen escape components (LEEi)

LEEi = SOEi - LFEi 3.12

Large intestine escape of rumen microbial components (LEMi)

LEMi = SOMi - LFMi 3.13

Large intestine escape of endogenous components (LEGi)

LEGi = SOGi - LFGi 3.14

Large intestine escape components (LEi)

LEi = LEEi + LEMi + LEGi 3.15

Large intestine microbial growth on fermented rumen escape components (LMEi)

LMEi = LFEi * YMDi * LM_{η} 3.16

LMEN = LMEAAN + LMENPN 3.17

LMECP = LMEN * 6.25 3.18

LMEOM = LMECP+LMELI+LMESS+LMENDF+LMEFP+LMEMC 3.19

Large intestine microbial growth on fermented rumen microbial components (LMMi)

LMMi = LFMi * YMDi * LM_0 3.20

LMMN = LMMAAN + LMMNPN 3.21

LMMCP = LMMN * 6.25 3.22

LMMOM = LMMCP+LMMLI+LMMSS+LMMNDF+LMMFP+LMMMC 3.23

Large intestine microbial growth on fermented endogenous components (LMGi)

LMGi = LFGi * YMDi * LM_0 3.24

LMGN = LMGAAN + LMGNPN 3.25

LMGCP = LMGN * 6.25 3.26

LMGOM = LMGCP+LMGLI+LMGSS+LMGNDF+LMGFP+LMGMC 3.27

Large intestine microbial components (LMEi)

LMi = LMEi + LMMi + LMGi 3.28

Large intestine digested rumen escape components (LDEi)

LDEi = LFEi - LMEi 3.29

Large intestine digested rumen escape components (LDEi)

LDMi = LFMi - LMMi 3.30

Large intestine digested rumen escape components (LDEi)

LDGi = LFGi - LMGi 3.31

Large intestine digested rumen escape components (LDEi)

LDi = LDEi + LDMi + LDGi 3.32

Large intestine output components (LOi)

LOi = LBi + LMi 3.33

Large intestine nitrogen balance (LNB)

LNB = LFN - LMN 3.34

Metabolism compartment

Small intestinal digestible amino acid requirements for

- N in skin, skurf and hoofs (SN):

SDAANSN = (0.2 * $LW^{0.6}$)/6.25 / 0.67 4.1

- Endogenous-N in urine (EUN):

SDAANEUN = (2.75 * $LW^{0.5}$)/6.25 / 0.67 4.2

- Endogenous-N in gastro intestinal tract (GN):

SDAANGN = (RGAAN - SDGAAN) / 0.80 4.3

- N in foetal and maternal tissues (YN):

SDAANYN =
(34.375*EXP(8.5357-13.1201*EXP(-0.00262*GDAY)-0.00262*GDAY))/6.25 / 0.50 4.4

- N in body protein (BN):

Energy balance

- Fat and protein corrected milk production:

FPCM = (0.337+0.116*%FAT+0.06*%PROT)*KGM 4.5

- NEL for maintenance:

NELMAIN = $41.34*LW^{0.75}$ * 1.65 4.6

- NEL for milk production:

NELMILK = (440*FPCM+0.7293*$FPCM^2$) * 1.65 4.7

- NEL balance:

NELBAL = NEL - NELMAIN - NELMILK 4.8

For animals in a positive energy balance

BN = NELBAL * 0.0174 / 6.25 4.9a

SDAANBN = BN / 0.50 4.10a

UNBN = SDAANBN - BN 4.11a

For animals in a negative energy balance

BN = NELBAL * (0.0174/0.8) / 6.25 4.9b

SDAANBN = BN * 0.80 4.10b

UNBN = SDAANBN - BN 4.11b

- N in milk (LN)

- *SDAAN available for milk protein production*

SDAANLN = SDAAN -
(SDAANSN+SDAANEUN+SDAANGN+SDAANBN+SDAANYN) 4.12

- *Ratio between SDAAN and NEL for milk production*

SDLNNEL = SDAANLN/NELMILK 4.13

- *Efficieny of SDAAN for milk protein production*

YL = 1.6085 - 100.1 * SDLNNEL 4.14

LN = SDAANLN * YL 4.15

Excretion of Nitrogen in urine

UN = SDAANEUN + (1- 0.67) * SDAANSN + (1- 0.20) * SDAANGN + UNBN+
(1- 0.50) * SDAANYN + (1- YN) * SDAANLN + SDNPN - RGNPN + RNB + LNB 4.16

CHAPTER 8

GENERAL DISCUSSION

Introduction

Optimising feed utilisation and production in dairy cows requires knowledge about the amount and nature of nutrients needed to achieve a certain production level (requirement or utilisation of nutrients), and about the capability of feedstuffs to yield those nutrients (supply). In feed evaluation systems those two aspects are separately described. Nutrient utilization mainly depend on animal characteristics (weight, production level) whereas nutrient supply is determined by feedstuff characteristics (chemical composition, digestion coefficients in rumen and intestine). Requirement and supply are expressed in comparable units, to enable the optimisation of dairy cow rations.

In early protein evaluation systems this unit was changed from an easy analysis of the N in feed (CP), which was hardly linked to the protein digestion process in the animal, to a unit that described the apparently digestible crude protein (DCP). The DCP-supply was calculated from the apparent digestibility of CP in feedstuffs measured in wethers fed at maintenance level of feeding. The DCP requirements for maintenance and milk production for dairy cows at higher production levels were established and validated in production trials (Frederiksen, 1931; Frens & Dijkstra, 1959). With increasing knowledge of the protein digestion and metabolism, limitations of the DCP-system became apparent, which were mainly the result of neglecting protein degradation and microbial protein synthesis in the rumen (Tamminga, 1986).

Recently introduced protein evaluation systems follow the complex processes of protein digestion and metabolism more closely than the DCP-system, and predict the supply and requirements of total amino acids absorbed in the small intestine (Chapter 3). The supply of amino acids is the sum of absorbed amino acids from protein escaping from rumen degradation and microbial synthesis in the rumen. Escape protein was initially assumed to be constant, but with development of *in vitro* and *in sacco* techniques different escape values were introduced for different feedstuffs or qualities within a feedstuff. The estimation of microbial protein was initially based on OM apparently digested in the rumen, which was assumed to be a constant proportion of total ME or DOM (ARC, 1984) for reasons of simplicity. This constant proportion was subsequently substituted by a correction for energy that is not available for rumen microbes (fat, fermentation products and escape protein and escape starch) for individual feedstuffs (Vérité *et al.*, 1987; Tamminga *et al.*, 1994). Amino acids that are needed to compensate for metabolic losses of amino acids in enzymes, mucus and intestinal cell walls are in most systems included in the requirement part of the system. An exception is the DVE-system in which the protein value of each feedstuff is corrected for necessary replacement of metabolic faecal loss of amino acids that is assumed to take place during digestion of this particular feedstuff.

In the CP and DCP-system protein requirements were related to metabolic body weight and milk production. Newly developed systems describe protein requirements in more detail. Requirements are made up by the sum of absorbed amino acids used for different

metabolic processes in the animal. The efficiency by which those amino acids are used varies between processes, and can also be dependent on the supply of other nutrients (like the DVE to NEL ratio in the DVE-system; Chapter 3).

Further development of protein evaluation

The complexity of current protein evaluation systems brings about two major challenges for further research. Protein values in the current systems are measured with complex and laborious methods (nylon bag, mobile nylon bag), which cannot be used in practise for a rapid screening of feedstuffs varying in quality. The first challenge is therefore the development of easy and cheap methods or regression equations to make a rapid estimate of protein values of feedstuffs. Those methods also can be used to increase the accuracy of the current methods or serve as alternatives. Because protein evaluation systems are build up by integrated parts, which are validated as a whole, changing one parameter value will affect other parameters. For instance changing the calculation rules towards an increased supply of digestible amino acids, will have to result in a lower efficiency by which those amino acids are used for milk production because the calculation rules per se have no effect on milk protein production. The alternative methods should therefore be validated against the standard methods or calculation rules, regardless whether the alternative resembles the *in vivo* situation more closely. In the first section of this chapter the background of current methods used to estimate DVE values of feedstuffs and possible alternatives are discussed.

The second challenge is to further develop systems to improve the estimation of N-excretion in milk, urine and faeces and enable the improvement in N-utilisation. This demands for further scientific research towards (more sophisticated) methods and models that more closely describe the *in vivo* situation. Two possibilities are discussed in the second section of this chapter: a more mechanistic and dynamic approach of protein evaluation and refinement of the system towards individual digestible amino acids.

The last section in this Chapter deals with the possibilities to improve N-utilisation in dairy cows by feeding management.

METHODS TO ESTIMATE PROTEIN VALUES AND ALTERNATIVES

Escape protein fraction

Current method

In the DVE-system the escape protein fraction (E_{CP}) of feedstuffs are given in feedstuffs tables (CVB, 1994) or can be calculated with regression equations. Those values were calculated from degradation characteristics (soluble and undegradable fraction and degradation rate of the potentially degradable fraction) measured with the nylon bag

method combined with an appropriate passage rate (Ørskov & McDonald, 1979). Although the nylon bag method is used as a standard technique, the methodology introduces many sources of variation. The technique was first developed for evaluation of protein rich concentrate ingredients, and later extended to other concentrate ingredients and roughages, which introduced even more sources of variation. These include sample preparation (homogenisation and drying method), sample weight in relation to bag size, bag pore size, incubation procedure (washing method to determine the soluble fraction in relation to particle loss and microbial contamination; determination of the undegradable residue), model to calculate degradation characteristics and animal factors (diet, production level, number of animals). The nylon bag technique is widely discussed elsewhere (Lindberg, 1985; Nocek, 1988; Michalet-Doreau and Ould-Bah, 1992; Van der Koelen *et al.*, 1992).

Only part of this variation can be reduced by standardisation of the methodology. Despite attempts in workshops to describe general rules for standardisation, ring tests revealed a large variation in methodology and results of the nylon bag method (Evans & Cottrill, 1980). Even a well defined method of analysis of N in feedstuffs showed a large variation (Madsen & Hvelplund, 1994). Recently proposed adaptions are the correction for loss of particles by washing through filter paper (Madsen & Hvelplund, 1994), reduction of microbial contamination by 'stomacher' treatment of residues (Ould-Bah *et al.*, 1988) and inclusion of standard samples in each incubation series (Vérité *et al.*, 1990). Although these adaptions will result in a better reproducibility of the technique, it becomes more laborious and complex. Despite its limitations, the nylon bag method is still regarded as the best available method to estimate rumen degradation characteristics of CP of feedstuffs, and therefore widely accepted.

An example of variation in degradation characteristics of CP is given in Table 1. This table gives the comparison between values measured in a single experiment (Chapter 6) and tabulated values used for the N-flow model calculations in Chapter 7, which are a compilation of literature values (Tamminga *et al.*, 1990) and unpublished data. Although the tabulated and measured degradation characteristics show sometimes large differences, the differences in calculated escape fractions are rather small, except for beetpulp and maize silage. The differences for beetpulp are probably caused by the large difference in sugar content between both sources. In general, a lower soluble fraction or undegradable fraction was compensated by a higher potentially degradable fraction, but also by a higher degradation rate.

In the DVE-system passage rate is assumed to be constant, with a higher value for concentrate feedstuffs than for roughages (6 and 4.5%/ h, respectively). This constant is an average of several published values (Vérité *et al.*, 1987). Hindle *et al.* (1995) concluded from a study with palmkernel expeller that also within concentrate feedstuffs the assumption for a constant passage rate seems to be unlikely. Passage rates can be measured using markers that are applied to the feed. From the concentration curve of the marker in the duodenum or faeces, passage rate can be calculated. Methodological

sources of variation in passage rate are type of marker, site of sampling, number of samples and mathematical model (Owens & Goetsch, 1986; Shaver *et al.*, 1986). In several studies it was shown that passage rate was not constant but increased with increasing feed intake level and roughage to concentrate ratio in the diet. This approach was followed for the N-flow model (Chapter 7).

Table 1. Comparison of degradation characteristics of crude protein and starch and NDF observed in an experiment (Chapter 6) and values used in the model (Chapter 7; tabulated) of concentrate feedstuffs and roughages

		Crude protein				Starch				NDF	
Feedstuff		U (%)	S (%)	k_d (%/h)	E (%)	U (%)	S (%)	k_d (%/h)	E (%)	U (%)	k_d (%/h)
Beetpulp	obs.	6	51	6.3	27					7	7.8
	tab.	6	12	5.2	50						
Maizeglutenfeed	obs.	3	48	4.9	30	0	31	12.5	23	5	3.3
	tab.	6	45	5.2	32	0	62	10.2	14		
Maizefeedmeal	obs.	5	39	2.6	37	0	47	7.0	25	8	2.4
	tab.	2	28	7.8	32	0	34	5.3	35		
Palmkernelexpeller	obs.	10	23	1.3	65					26	4.0
	tab.	7	9	3.5	60						
Soyabeanhulls	obs.	4	29	4.3	43					4	2.7
	tab.	8	25	5.6	43						
Soyabeanmeal	obs.	0	14	5.7	44					1	3.2
	tab.	0	7	8.0	40						
Grass silage	obs.	7	54	7.4	22					12	3.7
	tab.	5	54	6.5	22						
Maize silage	obs.	18	77	1.2	22					30	1.5
	tab.	9	57	2.3	32						

[1] U = undegradable fraction; S = soluble fraction; k_d = degradation rate of potentially degradable fraction (D = 100 - S - U); E = escape fraction $E=U+D\ (k_p/(k_d+k_p))$, with k_p concentrates = 6.0 %/h and k_p roughages = 4.5 %/h.

Alternatives

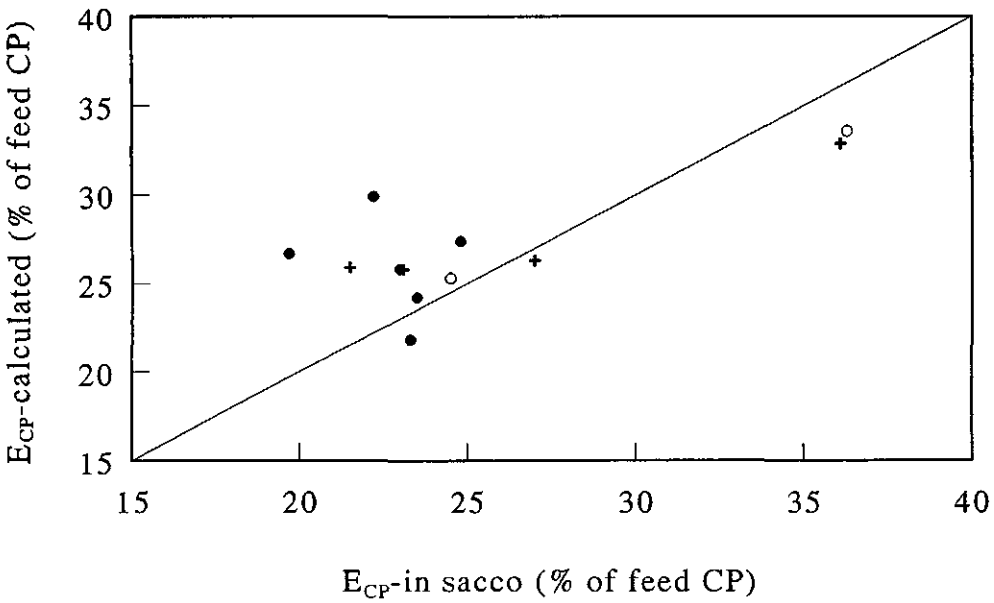
Many alternatives for the nylon bag method to estimate E_{CP} have been developed, and these will only be briefly discussed here. Methods include protein solubility and *in vitro* digestion using enzyme cocktails. Solvents proposed to measure protein solubility are hot water, borate-phosphate buffer, ethanol, NaCl solution, McDougall's artificial saliva and Burroughs' mineral mixture (Nocek, 1988, Van Os and Van Vuuren, 1991). Although these methods estimate protein solubility, they were found to be unsuited to estimate E_{CP} in general. Reasonable relationships can only be expected within a feedstuff or group of similar feedstuffs (De Boever *et al.*, 1984; Madsen and Hvelplund, 1985). The main reason for lack of relationship is that protein fractions in feedstuffs have a different susceptibility concerning solubility and degradability, because of differences in protein structure and configuration (Nocek, 1988).

In vitro methods using enzymes can be separated in methods using microbial enzyme cocktails (mostly from Streptomyces griseus, Aspergillus oryzae) or purified proteolytic enzymes (trypsine, chymotrypsine, pepsine). These methods were used to study the dynamics of the degradation (degradation rate) and total degradability. Of these the cocktail from Streptomyces griseus seems to be most promising. Several authors observed a good relationship between E_{CP} obtained with this method and with *in sacco* or *in vivo* methods (Van Os and Van Vuuren, 1992), but this was not the case for the underlying degradation characteristics. The undegradable fraction and degradation rate measured with the *in vitro* method was higher than those measured with the *in sacco* technique (Cone *et al.*, 1994a), which is probably due to the limited and in time declining activity of enzymes. The method needs further development and standardisation before practical use is possible. Different authors advise different adaptions to the original method: pre-incubation with carbohydrate degrading enzymes (amylase, cellulase), number of incubation times to estimate E_{CP}, optimal pH, amount of sample (Aufrère *et al.*, 1991; Assoumani *et al.*, 1992; Cone *et al.*, 1994a).

Regression equations by which E_{CP} can be estimated from the chemical composition of the feedstuff can be regarded as alternatives for nylon bag incubations. For fresh grass, grass silage and grass hay equations are available that explained 73 to 81% of the variation in E_{CP} (Chapter 2). However, using recently obtained data of fresh grass (including those of Chapter 4), Van Vuuren (1993) showed that the equation frequently underestimated E_{CP} of grass, which was different for diploid and tetraploid grasses, and presented a new equation. Since then even more data became available (Kappers and Valk, 1994). For use in the DVE-system the equation for grass silage given in Chapter 2 was changed to include 5% of the soluble fraction in E_{CP} (Tamminga *et al.*, 1994). A comparison between E_{CP} estimated with the new equation and measured in recently carried out nylon bag experiments (De Visser *et al.*, 1993 and unpublished; Van Straalen, unpublished; Veen, unpublished) showed a large difference for some samples (Figure 1). Data from Veen (unpublished) were corrected by using a standard soyabeanmeal as

reference sample. For clover a constant value of E_{CP} (30%) was adopted in the DVE-system, but in the experiment mentioned in Chapter 4 a variation between 32% to 40% was measured. Attempts to explain this variation by chemical composition have not been carried out yet. In conclusion, further research is needed to increase the accuracy by which the E_{CP} of roughages can be estimated from chemical composition.

Figure 1. Relationship between escape protein (E_{CP}) in grass silage estimated according to the DVE-system and E_{CP} measured *in sacco* (data from De Visser *et al.*, 1993 and unpublished (●), Van Straalen, unpublished (○) and Veen, unpublished (+)).



Digestibility of E_{CP}

Current method

Techniques to measure the digestibility of E_{CP} actually measure the indigestible fraction (I_{CP}). In the DVE-system those data for concentrate ingredients were obtained from

mobile nylon bag experiments (Chapter 2). Because this technique includes a rumen incubation step, the limitations for the rumen nylon bag method also are applicable to the mobile nylon bag method. Methodological aspects that need further study are the preparation of rumen residues for intestinal incubation (drying, grinding method), simulation of abomasal digestion (concentration and duration of pepsine-HCl incubation), influence of large intestinal digestion, and above all washing method of intestinal residues (Chapter 5). For roughages no data obtained with mobile nylon bag studies were available, so in the DVE-system I_{CP} was assumed to be equal to the residue after prolonged rumen incubation (generally 14 days). Several studies indicated that time of rumen incubation had only limited effects on I_{CP}, which would imply that each feedstuff has a constant I_{CP} fraction (De Boer *et al.*, 1987; Hvelplund *et al.*, 1992; Chapter 5). This assumption was used in the N-flow model described in Chapter 7.

Alternatives

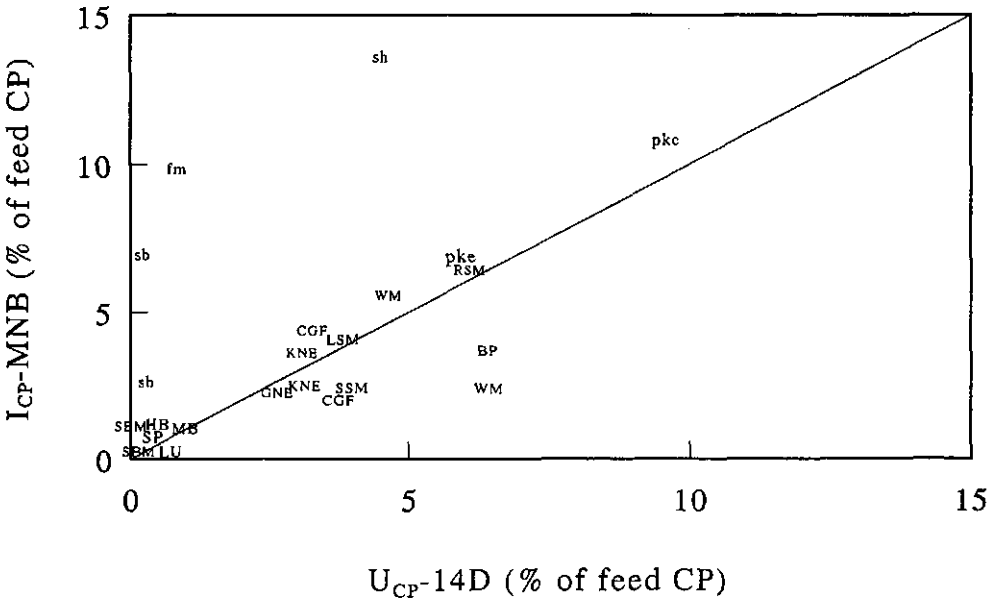
Several methods were proposed as alternatives to the mobile nylon bag method to measure the I_{CP} of feedstuffs: analysis of ADIN, prolonged rumen incubation, *in vitro* degradation, and infusion in intestine or digestibility in rats or hamsters. Because tabulated values for concentrate ingredients were obtained with the mobile nylon bag method, these methods are compared to the mobile nylon bag method.

The major advantage of the ADIN method is that it can be used in routine laboratory analysis of feedstuffs, and no fistulated animals are needed. However, the relationship between ADIN and I_{CP} according to the mobile nylon bag method is generally poor (Chapter 5 and 6, Peyraud *et al.*, 1988; Aufrère *et al.*, 1990).

The prolonged rumen incubation method has the advantage that no duodenal cannulated animals are required. This method can not be used in routine laboratories because still rumen cannulated animals are needed. For roughages a reasonable relationship between the rumen residue and I_{CP} measured with the mobile nylon bag method was observed (Chapter 5). For concentrate ingredients this relationship is shown in figure 2 (data from Tamminga and Ketelaar, 1988; Veen *et al.*, unpublished and Chapter 6). When excluding the most extreme feedstuffs (palmkernel, feathermeal, soyabeanhulls and soyabeans), the following relationship was observed: $I_{CP} = 0.159 + 0.886 \times U_{CP}$ ($R^2 = 0.76$; n=20).

Different procedures to measure I_{CP} with an *in vitro* method were developed, using a pre-incubation with pepsine HCl to mimic abomasal digestion, followed by an incubation with pancreatin (Vicini *et al.*, 1983; Antoniewicz *et al.*, 1992) or trypsine and chymotrypsin (Setälä *et al.*, 1984) to simulate intestinal digestion. With these methods duodenal cannulated animals are not required, but rumen residues are still needed. With roughages a reasonable relationship between I_{CP} measured with the Antoniewicz *et al.* (1992) method and the mobile nylon bag method was observed (Chapter 5). Only a few data for concentrate ingredients are available (Antoniewicz *et al.*, 1992, Veen *et al.*, unpublished). In figure 3 the relationship is presented. Especially feathermeal and formal-

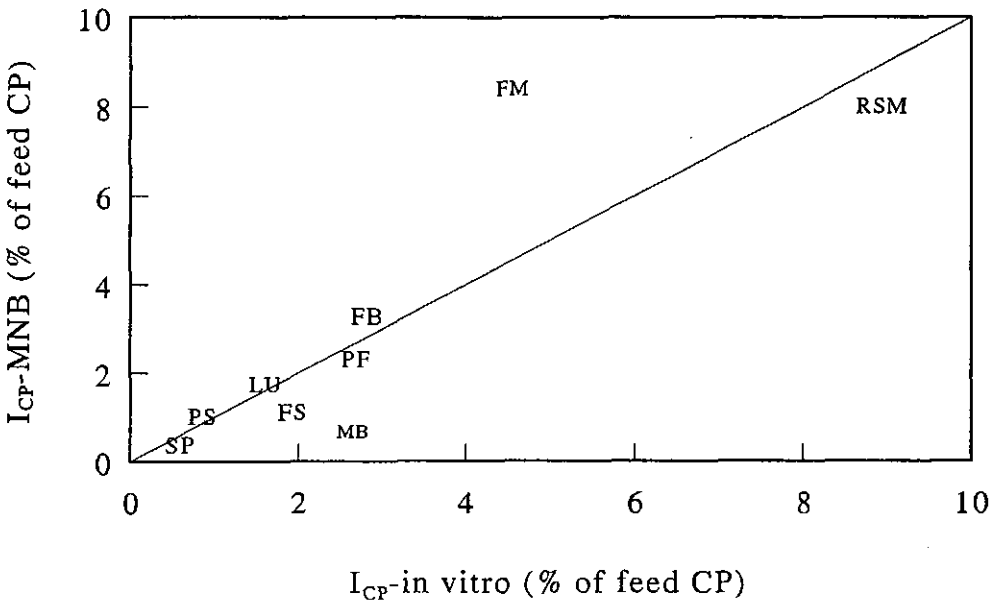
Figure 2. Relationship between indigestible CP measured with the prolonged rumen incubation (U_{CP}-14D) and mobile nylon bag method (I_{CP}-MNB) for concentrate ingredients (data from Tamminga and Ketelaar 1988; Veen, unpublished and Chapter 6). BP=beetpulp; CGF=cornglutenfeed; fm=feathermeal; GNE=groundnut expeller; HB=horsebeans; HF=hominy feed; KNE = coconut expeller; LU=lupin; LSE=linseed expeller; MB=mervobest (protected soyabeanmeal) pke=palmkernel expeller; RSM=rapeseedmeal; sb=soyabean; SBM=soyabeanmeal; sh=soyahulls; SP=soypass (protected soyabeanmeal); SSM=sunflowerseedmeal; WM=wheat middlings. (Feedstuff codes in capitals are included in regression)



dehyde treated soyabeanmeal showed a large difference between the two methods. To draw conclusions of the possibility of this method as alternative for the mobile nylon bag method further research with more samples is needed.

Also an *in vitro* method with jejunal digesta was tested (Todorov & Girginov, 1991). The results were comparable to those obtained with mobile nylon bags in the intestine, but this method is not an alternative because duodenally cannulated animals are still required.

Figure 3. Relationship between indigestible CP measured with an *in vitro* method (I_{CP}-in vitro) and the mobile nylon bag method (I_{CP}-MNB) for concentrate ingredients (data from Antoniewicz *et al.* 1992; Veen, unpublished). FB=fieldbeans; FM=feathermeal; FS=fishmeal; LU=lupins; MB=mervobest (protected soyabeanmeal); PF=presscake fishmeal; RSM=rapeseedmeal; SP=soypass (protected soyabeanmeal)



With the infusion method feedstuffs or rumen residues are infused in the beginning of the small intestine and the increase in ileal protein flow or faecal excretion is measured (Schwarting and Kaufmann, 1978; Hvelplund *et al.*, 1985). Also this method requires cannulated animals. Although Hvelplund *et al.* (1985) and Todorov & Girginov (1991) observed a close agreement between the digestibility measured with infusion and mobile nylon bags, in general lower values were observed, probably due to increased endogenous excretion (Chapter 2).

Susmel *et al.* (1994) compared the *in vivo* true digestibility of CP in feed and rumen residues measured in hamsters with the digestibility measured with mobile nylon bags. Samples that were pre-incubated in the rumen showed a slightly lower digestibility than those not incubated in the rumen for both methods. Despite a correction for endogenous

contribution, the *in vivo* method resulted in a lower digestibility than the mobile nylon bag method and the relationship between both methods was poor.

As for E_{CP}, regression equations by which I_{CP} can be estimated from the chemical composition of the feedstuff serve as an alternative for nylon bag incubations. For fresh grass, grass silage and clover relationships were obtained, but with a limited number of data (Chapter 5).

Fermentable organic matter

Actual situation

In the DVE-system the FOM is calculated from the apparent DOM minus all the components from which is assumed that no energy becomes available for rumen microbes: E_{CP}, escape starch fraction (E_{ST}), fat and half of the fermentation products in ensiled forages (Tamminga *et al.*, 1994). In this approach it is assumed that apparently undigested feed CP and starch and fat is compensated by OM fermented in the hindgut. Two attempts were made to validate this assumption using the predicted averages of datasets 1 to 4 (111 treatments) from the N-flow model (Chapter 7). The FOM calculated according to the DVE-system was on average 84 g/kg DM ration lower than the FOM predicted according the N-flow model from the degradation characteristics of the components of the OM. On the other hand, the average predicted OM absorbed in the large intestine (68 g/kg DM ration) was larger than the sum of undigested escape CP (11 g/kg DM), undigested escape ST (7 g/kg DM) and undigested fat (6 g/kg DM). This would indicate that the FOM in the DVE-system is overestimated by 44 g/kg DM on average. Further research is needed to validate this assumption.

The E_{CP} and E_{ST} are calculated from nylon bag incubations in the rumen. This technique is already discussed. Also for degradation characteristics of starch large differences can occur between tabulated (as used in Chapter 7) and single measurements (Chapter 6; see Table 1). In contrast to protein, it was assumed that 10% of the soluble starch measured with nylon bags was not fermented in the rumen and belonged to the escape starch (Tamminga *et al.*, 1994). This was done to correct for differences between observed *in vivo* starch flow in the duodenum and calculated flow from *in sacco* experiments (Nocek and Tamminga, 1991). This difference could possibly be due to overestimation of the soluble fraction with the nylon bag technique or to incorporation of starch in microbial cells. Studies showed that there was a large difference between starch that disappeared from nylon bags after washing and starch that was soluble in water (De Boever *et al.*, 1995). The E_{ST} value in the DVE-system was further corrected for the negative effect of pelleting concentrate feeds (corrected $E_{ST} = 0.75 \times E_{ST}$). Due to lack of data, this correction was assumed to be equal for all feedstuffs. Recent research showed that the effect of pelleting is smaller than was assumed (correction factor 0.875 instead of 0.75) (Goelema, unpublished).

Alternatives

In the calculation of FOM for E_{CP} and E_{ST} nylon bag data are required. Alternatives for estimating E_{CP} are discussed previously. Cone *et al.* (1989) and Cone and Vlot (1990) tested several *in vitro* methods for E_{ST} based on fresh rumen fluid and enzymes (α-amylase, pancreatine and amyloglucosidase). Although these methods generally showed the same ranking of feedstuffs, they concluded that enzymatic methods showed only limited possibilities to estimate E_{ST} measured with fresh rumen fluid. The relationship between *in vitro* and *in sacco* starch degradation was studied combining data from Cone *et al.* (1989), Cone and Vlot (1990) and Tamminga *et al.* (1990). Generally less than 50% of total variance in the soluble or effectively fermented starch measured *in sacco* could be explained by the disappearance after incubation with α-amylase, pancreatine, fresh rumen fluid or extract of rumen fluid (given by Cone, 1989), except for the relationship between soluble starch and the disappearance after incubation with rumen fluid extract (R^2=0.66, n=15).

The FOM can also be estimated directly by *in sacco* or *in vitro* methods. Only limited information on total OM degradation measured *in sacco* is available from the literature because studies were mainly focused on protein, starch or cell walls. The estimation of FOM from degradation characteristics requires an assumption for the passage rate. Different components of the OM have different passage rates, and the cell wall fraction probably does not follow first order kinetics (Tamminga, 1989; Chapter 7), which makes an assumption on passage rate of total OM more difficult. A comparison between FOM calculated from degradation characteristics of total OM and according to the DVE-system of some concentrate ingredients and roughages is given in Table 2. For some feedstuffs there was a close agreement between both ways to estimate FOM, but for other there was a large difference (especially soyabeanhulls). Van Vuuren (1993) observed for fresh grass that the difference between FOM according to the DVE system and FOM calculated from *in sacco* measurements increased with decreasing CP content, possibly due to a change in passage rate in the rumen and large intestinal fermentation.

Another approach to estimate FOM from nylon bag data is to calculate the sum of fermented components of OM (CP, ST, NDF, fat, FP and miscellaneous components), from the degradation characteristics of those components, as was done in the N-flow model presented in Chapter 7 (Table 2). Besides the problems associated with the estimation of passage rates, also assumptions on the behaviour of the miscellaneous components in the rumen are subject to discussion (Chapter 7).

Cone *et al.* (1994b) carried out several *in vitro* methods (fresh rumen fluid, cell-free rumen fluid and enzyme cocktail) and compared the results to the FOM calculated from data obtained with the nylon bag method. They concluded that all these methods could be used to estimate FOM measured *in sacco*, with the exception of feedstuffs high in fat or starch. However, *in vitro* methods based on rumen fluid are not available for routine

Table 2. Degradation characteristics of OM observed in an experiment (Chapter 6) and comparison of different approaches of calculation of fermentable organic matter (FOM)

	Organic matter[1]					
Feedstuff	U (%)	S (%)	k_d (%/h)	FOM[2]	FOM[3] (g/kg DS)	FOM[4]
Beetpulp	3	42	8.3	770	708	634
Maizeglutenfeed	3	32	4.3	663	599	624
Maizefeedmeal	4	39	3.2	588	598	584
Palmkernelexpeller	19	17	3.9	453	444	460
Soyabeanhulls	3	16	3.2	686	570	623
Soyabeanmeal	1	29	6.1	585	640	473
Grass silage	10	29	4.2	584	555	624
Maize silage	15	41	2.1	546	545	667

[1] U = undegradable fraction; S = soluble fraction; k_d = degradation rate of potentially degradable fraction (D = 100 - S - U)

[2] FOM calculated according to DVE-system (FOM = DOM - ECP - EST - FAT - FP x 0.5)

[3] FOM calculated from degradation characteristics of OM (= S + D x $k_d/(k_d+k_p)$), with k_p arbitrarily assumed to be 4.5 %/h for concentrates and 3.5 %/h for roughages;

[4] FOM calculated from degradation characteristics of components of OM according to the model as described in Chapter 7 (sum of fermented CP, NDF, starch and sugar, fermentation products and miscellaneous OM); data from Table 1.

laboratories and are therefore no alternative. The undigested residue after 24 h obtained with the enzymatic method showed a good relationship with the FOM measured *in sacco* (Cone *et al.*, 1994b). Although the enzymatic method seems to be promising as an alternative, no conclusion can be drawn yet. No relationship between FOM according to the enzymatic method and FOM calculated as in the DVE system was given. Furthermore, no information was available about individual concentrate ingredients because only roughages and compound feeds samples were included in their study.

Another way to estimate FOM *in vitro* is the time related gas production test (TRGT) (Beuvink, 1994). In this method the *in vitro* gas production is measured and gas production data are fitted with a modified Gompertz model. The parameters estimated with this model (maximum gas production rate, lag phase, time at maximal gas production and total gas production) are difficult to compare with those obtained from the exponential model generally used to fit nylon bag degradation curves (U, S and k_d). For maize products there was a close agreement between the (1-U) fraction of OM and the total gas production, but not for the other parameters. Because this method is relatively easy to carry out it seems to be a promising method to estimate FOM. However, before it

can be used for routine analysis further development, standardisation and validation is required.

MODELLING OF PROTEIN EVALUATION

The DVE-system was introduced to accurately estimate milk protein production and in the meanwhile prevent unnecessary N losses. The system was based on other modern protein evaluation systems, which enabled a quick and successful introduction. Although the newly developed system has distinct advances over the DCP-system, still a number of approximations appeared necessary (Tamminga *et al.*, 1994). These include amongst others the factor 1.11 to correct digestible escape protein, the amino acid content in escape protein, the estimation of the fermentable organic matter from DOM, the use of a fixed passage rate and efficiency of microbial protein synthesis, the lack of effect of diet composition on the degradation rate, the estimation of endogenous excretions with the faeces and the effect of large intestine fermentation on the measurement of digestible protein by the mobile nylon bag method. Although with the DVE-system a reasonable estimation of milk protein production could be obtained (Chapter 3), it systematically underestimated N excretion with the faeces and overestimated urinary N-excretion (Van Straalen, unpublished observations).

To improve estimations of N-excretions in dairy cows by protein evaluation systems and to overcome the above mentioned shortcomings, further development in two ways will be discussed: 1: incorporation of protein evaluation into whole animal nutrient digestion and metabolism; and 2: extension of protein evaluation towards individual amino acid evaluation.

Protein evaluation and whole animal nutrient digestion and metabolism

Several models that describe nutrient digestion and metabolism of varying complexity have been developed. The most complex are dynamic, deterministic and mechanistic models, in which nutrient flows between pools are described by differential equations, representing Michaelis Menten or first order mass action kinetics (Baldwin *et al.*, 1987; Danfaer, 1990; Dijkstra, 1993). These models are mainly meant for scientific purposes to improve concepts of digestion and metabolic processes and design of experiments. A more practical approach was used in the CNCPS model (Russell *et al.*, 1992) and in the N-flow model (Chapter 7). Those models can be described as static, deterministic and mechanistic when concerning the prediction of protein supply. Protein requirements are mainly based on empirically derived equations. The N-flow model was developed to predict N excretions. Besides estimation of N excretions with milk, faeces and urine, it can also be used to estimate protein values of feedstuffs under various physiological conditions. For formulation of diets it is of interest whether the ranking of feedstuffs

according to protein value predicted by the N-flow model is different from the ranking according to DVE, and the effect of the more deterministic approach on protein values of feedstuffs.

The basis for the calculation of digestible escape protein are equal for both the DVE-system and the N-flow model (rumen and intestinal degradation parameters measured with nylon bag incubations). The major differences are that in the N-flow model passage rate depends on feed intake, amino acid content of escape protein is equal to that in the feed, and small intestinal amino acid digestibility is corrected for large intestine fermentation and the amino acid content of escape protein. Furthermore in the N-flow model, digestible microbial protein is calculated from the fermentation of each component of the OM with a variable efficiency (dependent on degradation rate and type of microbes). The fermentability of each component of the OM was calculated from degradation characteristics measured with nylon bag incubations and for each component of OM a passage rate dependent on feed intake. The endogenous intestinal excretions were calculated from the passage of OM in the beginning of the small intestine.

Figure 4. Digestible protein in some feedstuffs, calculated according to the DVE-system (label DVE) and according to the model (Chapter 7) at feed intake levels of 10 and 24 kg DM/d (label 10 and 24 respectively). TP=tapioca; MA=maize; BP=beetpulp; SH=soyabeanhulls; SBM=soyabeanmeal; FM=fishmeal

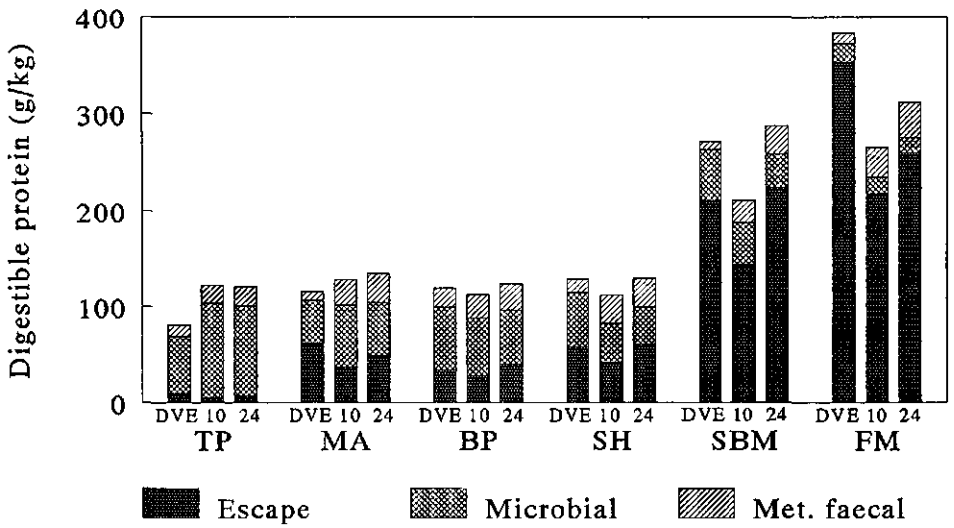


Figure 4 shows the difference between the estimated protein value in the DVE-system and according to the N-flow model at two feed intake levels (10 and 24 kg DM/day) for some concentrate ingredients (differing in composition and degradation rate). In the N-flow model, the passage rate increased at a higher feed intake level, which resulted in a higher escape fraction and higher digestion of escape protein in the small intestine, the latter because of a lower correction for large intestine fermentation. This higher intake, however, also resulted in a lower microbial protein synthesis due to a lower fermentation of OM in the rumen. At a higher feed intake level the endogenous intestinal excretions increased because of an increase in OM flow in the beginning of the small intestine. The net effect of feed intake level on the protein value depended on the protein content of the feedstuff. In feedstuffs with a low protein content, the increase in escape protein is compensated by a decrease in microbial protein, while in feedstuffs with a high protein content the increase in escape protein was higher than the decrease in microbial protein synthesis. The difference in protein value between the N-flow model and the DVE-system was therefore only significant in feedstuffs with a high protein content. The protein value in the DVE-system was comparable to the value calculated by the N-flow model at a high feed intake level, and higher at a low intake level. This implies that at a lower production level the actual protein supply is lower than calculated by the DVE-system, which is in contrast to the higher efficiency at which DVE is used for milk protein production at lower production levels compared to higher levels (Subnel *et al.*, 1994).

Extension of protein evaluation towards individual amino acid evaluation

As for total protein, evaluation systems for individual amino acids, starting with the first limiting amino acids, should be made up by a supply and requirement part. To identify first limiting amino acids for milk production several methods were used in literature: comparison of amino acid profiles of feedstuffs or digesta to that of milk protein, measuring effects of infusions of amino acids in the gastrointestinal tract on amino acid concentration in blood plasma or on milk protein production or the change of amino acid concentration in the blood during the lactation (DePeters and Cant, 1992; Meijer *et al.*, 1995). The sequence and extend of limiting amino acids depends on the dietary composition. Most infusion experiments identified methionine and lysine to be first limiting or co-limiting for milk protein production (Schwab *et al.*, 1992a; Rulquin and Vérité, 1993; Guinard and Rulquin, 1994). However, the importance of limiting individual amino acid was doubted by Choung and Chamberlain (1995) who concluded from infusion experiments that products of protein hydrolysis (peptides) are possibly of higher importance for milk protein production than single amino acids.

For the estimation of individual amino acid supply the factorial approach of total protein should be followed. Assuming that total protein supply can be estimated with a certain accuracy, the reliability of the estimation of individual amino acid supply is further hampered by the lack of knowledge and/or large variation in the amino acid

profile of digestible escape, microbial and endogenous protein. For the limited number of feedstuffs that were studied, the effect of rumen incubation and intestinal digestion on the amino acid profile of escape protein varied between feedstuffs and experiments (see Chapter 6). More experiments are needed to obtain data for other feedstuffs and study the variation within feedstuffs. Because solubilisation showed to have a bigger effect on the amino acid profile than rumen incubation or intestinal digestion (Chapter 6), the profile of the insoluble residue can be a reasonable estimate of the profile of digestible escape protein. Clark *et al.* (1992) and Rulquin and Vérité (1993) observed a large unexplained variation in the amino acid profile of microbial protein, especially methionine. This profile determines largely the profile of total digestible protein and therefore the amino acid requirements. Systematic errors can easily be corrected for in the requirements, but other sources of variation, like variation between species of microbes, are more difficult to take into account (Rulquin and Vérité, 1993). To estimate amino acid requirements the profiles of milk, body and endogenous protein and the respective efficiencies are required. Milk protein has a rather constant amino acid profile, but for body protein a larger variation was observed (Ainsli *et al.*, 1993) whereas for endogenous protein hardly any information is available (Ørskov *et al.*, 1986). Although the knowledge about the utilisation of amino acids is increasing (Armentano *et al.*, 1994; Reynolds *et al.*, 1994), this information is yet to scarce to incorporate into a practical evaluation system.

As a first step towards amino acid evaluation in ruminants, Rulquin *et al.* (1993) proposed a system based on an extensive literature review. In their approach both the content in feedstuffs and the requirements of digestible methionine and lysine were presented as proportion of total PDI. The supply of methionine (MetDI) and lysine (LysDI) was calculated using the amino acid profile of the feed and assumed profiles for microbes and endogenous protein. The predicted amino acid flow in the duodenum was subsequently corrected for differences from the observed amino acid flow. Requirements were empirically derived from the relationship between MetDI and LysDI and milk protein production. This resulted in estimated optimum levels of 2.5% MetDI and 7.3% LysDI in PDI and minimum levels of respectively 2.0% and 6.8%.

Because of differences between the PDI- and DVE-system (Chapter 3) those values can not directly be translated towards the DVE-system. However, the basic principles and assumptions of the calculation of amino acid supply can also be used to develop the DVE-system towards a system for digestible amino acids. Requirements should be established by relating milk protein production to the calculated amino acid supply using historical data, as was carried out for total DVE (Chapter 3; Subnel *et al.*, 1994).

Reduction of N-loss by feeding management

In The Netherlands dairy husbandry is held responsible for a large part of N-pollution of the environment. Emission of ammonia contributes to deposition of 'acid equivalents' and loss of nitrate by leaching and surface runoff can result in higher nitrate concentration in soil water. Volatilization of ammonia and loss of nitrate can be substantially reduced by reduction of N input on the farm, followed by application of methods to reduce N losses, mainly in manure handling. The latter include covering of manure storages, restriction of the amount, method and season of N-application, and adaptions of stables (Aarts *et al.*, 1992; Korevaar, 1992).

It was estimated that in The Netherlands on average 80 to 85% of total N intake in dairy cows was excreted in urine and faeces (Aarts *et al.*, 1992; Korevaar, 1992; Tamminga, 1992). Only part of these losses can be reduced by increasing N-efficiency because of inevitable losses during digestion and metabolism. Van Vuuren and Meijs (1987) calculated a theoretical maximum efficiency (milk N/feed N) of 40 to 45%. To enable an increase of this N-efficiency by feeding management, sources of avoidable N-excretion should be identified. With special feeding management adaptions, these excretions can then be reduced.

The N-flow model (Chapter 7) was designed to give a detailed prediction of sources of N-excretion. This was done for data from feeding experiments (Dataset 1 and 4; Chapter 7; Table 3). For this database on average 27% of feed-N was excreted with milk, 37% with faeces and 35% with urine. Milk-N excretion was higher than estimated by Tamminga (1992) and Korevaar (1992) because in the current study mainly data from cows in early lactation were used. The loss of N in skin and endogenous urinary N can be regarded as inevitable (3% of N intake in our database). These losses are minimal maintenance requirements of the animal. Faecal excretion of undigested rumen microbial N, feed N and endogenous N, urinary excretion of digested NPN, N absorbed in the large intestine loss and N loss because of inefficiently used amino acid N for milk protein (69% of total N intake) can only partly be reduced by current knowledge of feeding management. Minimizing the absorption of N in the rumen (14% of N intake) offers the best possibility for reduction of N losses.

N-absorption in the rumen

The predicted N-loss in the rumen showed a large variation (Table 3). These losses can be reduced by optimising the ratio between fermentation of CP and OM. Van Vuuren *et al.* (1990) assumed an optimal N use in the rumen at a ratio of 25 g N per kg fermented OM, which agrees closely with the efficiency of microbial CP synthesis assumed in the DVE-system (150 g CP/kg FOM). This corresponds to an OEB-value in the diet of zero. Practical possibilities to lower the OEB value of a diet are the partial replacement of grass (silage) by corn silage and starch or cell wall rich concentrates (Valk *et al.*, 1990; Valk,

1994). To ensure optimal rumen fermentation and microbial growth at a low OEB value on daily basis, a further synchronisation of CP and OM on an hour to hour basis seems advisable. Practical methods to achieve this are frequent feeding and feeding total mixed rations in combination with matching degradation characteristics of CP and OM of dietary components (Van Vuuren, 1993).

At a given ratio between fermented CP and OM, the N-loss in the rumen can further be lowered by increasing the efficiency of microbial CP synthesis. In the N-flow model this efficiency is calculated from the degradation rate of the substrate and the type of substrate (starch or cell walls). Although this approach was not validated, it suggests that microbial protein synthesis can be improved and the rumen N-loss reduced by feeding diets high in non structural carbohydrate with a high degradation rate. However, the application of large amounts of non structural carbohydrates can result in a drop in rumen pH and the lack of structure can diminish rumen function. This will lead to a reduced fermentation of structural carbohydrates and even higher loss of N in the rumen.

Table 3: Estimated N-excretion in feeding trials (dataset 1 and 4; Chapter 7).

	Average	Percent of total excretion		
	g/d	average	minimum	maximum
milk	140	27	17	34
faeces	191	37	25	48
undigested feed	27	5	4	7
undigested rumen microbial	28	5	3	7
undigested endogenous	109	21	13	28
large intestine microbial	27	5	4	7
urine	184	35	17	55
endogenous urinary	16	3	2	4
rumen	71	14	-10	43
large intestine	36	7	5	9
digested NPN	34	7	4	9
metabolic faecal	-26	-5	-7	-3
milk	49	9	0	25
skin	1	0	0	0
body	2	0	-2	3
skin	1	0	0	0

Efficiency of milk protein production

Nitrogen lost with urine because of inefficient use of amino acids for milk protein production was predicted at almost 10% of total N intake. This efficiency can be increased by balancing nutrient supply to the mammary gland, resulting in an increase of first limiting nutrients. The efficiency of milk protein production was shown to be dependent on the ratio between protein (DVE) to energy (VEM) available for milk production and milk production level. Maximum milk protein production was estimated at DVE to VEM ratio's of 19.2 at 10 kg FPCM production and 17.6 DVE to VEM at 40 kg FPCM production (Chapter 3). The corresponding efficiencies can be calculated as 59 and 55 g milk protein per 100 g DVE available for milk protein production. Although a decrease in the DVE to VEM ratio at a given production level will result in a higher efficiency, total milk protein production will be lower. Efficiency of milk protein production can also be expressed as milk protein production divided by total DVE requirement:

efficiency = milk protein / (DVE for maintenance + DVE for milk)

For the DVE requirement for milk protein production the formula proposed by (Subnel *et al.*, 1994) can be used:

DVE for milk = 1.396 x milk protein + 0.00195 x milk protein^2 (in g/d)

With those two equations it can be calculated that the total DVE efficiency increases quickly with milk protein production from 0 to 500 g/d to a level of 58%, and remains fairly constant (57-59%) when milk protein production is further increased to 1500 g/d. The efficiency by which DVE available for milk production is used for milk protein decreases in the same traject (500 to 1500 g/d) from 67 to 59%. This means that in contrast to the efficiency of DVE available for milk production, the efficiency of total DVE is theoretically independent on milk protein production level. Other protein evaluation systems use a constant efficiency of the use of digestible protein for milk protein production (Chapter 3). For those systems an increase in the efficiency of the use of total digestible protein for milk protein production will be found with increasing milk production level.

A further increase in the efficiency of milk protein production can possibly be obtained by balancing amino acids within total protein or energy. Rulquin *et al.* (1993) observed an increase in milk protein yield and content when the methionine or lysine content in the PDI was increased. The marginal efficiency by which these extra amino acids are used for milk protein production is generally very low. However, extra first limiting amino acids increase the utilisation of non limiting amino acids and can lead to an increased efficiency of total CP utilisation. Based on limited number of data (Schwab *et al.*, 1992b;

Guinard *et al.*, 1994) an increase in the efficiency of total CP (milk CP/feed CP) between 0.3 and 2.6% (absolute) was calculated for treatments in which 8 to 10 g of methionine and/or 10 g of lysine was infused in the small intestine compared to the control treatment.

Endogenous faecal N-excretion

The N-flow model predicted that faecal endogenous N excretion was on average 21% of total N intake or 57% of total faecal N excretions. A large variation in this proportion was observed. However, the estimation of endogenous N excretion by the N-flow model was based on assumptions that are subject of discussion. The endogenous N excretion in the faeces is the result of the amount secreted in the small and large intestine, the absorption in the small intestine and fermentation in the large intestine. It was estimated that half of the endogenous N in the duodenum originated from the rumen and the other half was secreted in the intestine. In pigs a lower contribution of salivary and gastric secretion (10%) compared to small intestine excretions (17% pancreas, 9% bile, 57% small intestine wall, 7% sloughed cells) was observed (Schulze, 1994). However, in ruminants a higher contribution of endogenous N appearing before the small intestine can be expected because of the rumination activity. In Chapter 7 is was discussed that the estimated endogenous N flow in total duodenum N flow was comparable with values reported in literature. The negative apparent digestibility coefficient for endogenous N in the small intestine that was used in the N-flow model was much lower than the digestibility values from literature data (coefficients from 0.50 with sheep to 0.80 with pigs). These findings are in contrast with the fact that the predicted contribution of endogenous N in total ileal N (66%) was comparable with literature data from sheep (73%; Van Bruchem *et al.*, 1985) or pigs (75%; Schulze, 1994). About the effect of anti nutritional factors (ANF) on the apparent digestibility of endogenous N in the small intestine and the fate in the large intestine of ruminants no information is available. More research is needed to estimate the contribution of endogenous N to total faecal N.

Although the way endogenous N excretion in the N-flow model is estimated is subject to discussion, this excretion seems to be a major part of total N excretions. The relationship with OM passage in the small intestine as used in the N-flow model suggests that endogenous excretions in the faeces can be reduced by feeding diets with high fermentable OM (especially cell walls).

Digestibility of feed and microbial CP

Undigested feed and rumen microbial CP accounted for 10% of total N excretions and showed only a small variation. Because CP in roughages is generally less digestible than that in concentrates, this proportion will be higher when animals are fed more roughage with a lower quality. Concentrate feedstuffs generally have a high digestibility of total CP than microbial CP. It can be notified that increasing microbial synthesis in the rumen at

cost of escape CP from concentrates will result higher N excretions with the faeces. Microbial N also contains a higher proportion of NPN compared to escape feed N. This NPN is lost in the urine when not recycled to the rumen.

N absorption in the large intestine

The N absorption in the large intestine (on average 7% of total N excretion) is the balance between microbial fermentation of CP and production of microbial CP on basis of fermented OM. Changing this balance will only result in a decrease in N excretion if the absorbed N is recycled in the rumen. An increase in large intestine OM fermentation will result in a shift from N excretion in the urine towards the faeces. Because N in faeces is less readily available than N in urea, this will probably result in a lower loss of N by NH_3 volatilisation.

Conclusions

Further development of protein evaluation systems include the development of easy and cheap methods to estimate protein values of feedstuffs in practise and the incorporation of individual amino acid evaluation into a model for whole animal digestion and metabolism. Several alternatives for the nylon bag method to estimate E_{CP}, I_{CP}, E_{ST} and FOM have been developed, but further validation of those methods with more samples is needed before they can be satisfactorily used. These methods should be validated against the current methods, because they serve as an integrated part of the system.

Including protein evaluation into a whole animal digestion and metabolism model gives more detailed information on sources of N excretions. The efficiency of N utilisation by dairy cows can be increased by balancing CP and energy fermentation in the rumen and balancing individual amino acid and energy utilisation in the metabolism. Endogenous faecal N-excretion was a major factor contributing to total N-excretion. More research is needed to obtain information on the extend and factors influencing excretion.

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SUMMARY

During the last decades dairy husbandry in The Netherlands has developed towards an intensive production system, in which high production levels per cow are obtained. Recently there is an increasing awareness of the negative impact of such a system on the environment. In The Netherlands, the dairy husbandry is held responsible for a major part of the N pollution of air and soil water. Different methods to reduce N losses on farm level have been studied, including changes in diet composition. On average 80 to 85 percent of the N intake of a dairy cow was estimated to be excreted in urine and faeces. These losses can be reduced by closely matching the quantitative and qualitative supply of protein (Nx6.25) with the requirements of the animal. However, the protein evaluation system that was used until recently in The Netherlands (the DCP-system), showed limited possibilities to quantify the effects of diet composition on N losses, mainly because protein degradation and microbial protein synthesis in the rumen were not considered. Therefore, a new system based on recently acquired knowledge of protein digestion and metabolism was required.

The objective of this thesis was to develop a model that predicts N excretion in faeces, urine and milk. If N digestion and metabolism are described in more detail, sources of unnecessary N losses can be identified. The development of the model was simultaneously carried out with the development and introduction of a new protein evaluation system for practical use in The Netherlands (the DVE-system).

The development of the model was started by collecting and evaluating information from literature and unpublished experiments (Chapter 2 and 3).

Chapter 2 reviews recently obtained information on the degradation in the rumen and digestion in the small intestine of feed protein. Different methods are available to estimate protein degradation. The use of *in vivo* methods is limited by its indirect methodology and the requirement of duodenal cannulated animals. *In vitro* methods need further development to have a close agreement with *in vivo* protein degradation. The *in sacco* method appears at present the most reliable method to estimate protein degradation in the rumen, provided a standardized procedure is used. With this method protein can be separated in a soluble fraction, an undegradable fraction and an unsoluble, potentially degradable fraction with a degradation rate. Results of experiments carried out at ID-DLO (former IVVO-DLO) were presented in this way. From the degradation characteristics, the effectively escape fraction can be calculated, using assumptions for the passage rate. Data on effective protein escape of concentrate feedstuffs were collected from literature and corrected for laboratory differences. A large variation between feedstuffs was observed. Variation in degradation characteristics of forages could be related to composition and harvest date of the forage. Intestinal digestion of escape protein can be measured with *in vivo* or *in sacco* methods. Based on literature data, the mobile nylon bag method resulted in higher digestibilities than the infusion method. Rumen protein degradation and intestinal digestion can be manipulated by changing growing conditions (forage), processing (physical and chemical) and feeding management.

In several countries new protein evaluation systems have been developed and

introduced to replace the DCP-system. Although based on the same principles, systems vary widely in optimal protein concentration and escape fraction and accuracy of the estimation of milk protein production. In Chapter 3 a validation of protein evaluation systems (CP, DCP, PDI, AAT, AP, MP, AAS and DVE-system) was carried out using 15 production experiments with dairy cows. Only treatments that were deficient in protein according to at least one system were selected. Average production level was 989 g milk protein per day. Milk protein production was predicted from the protein supply and requirement in each system. The prediction error was smallest for the DVE-system, and increased in order of the CP, PDI, DCP, AP, AAS, AAT and MP-system. Predictions can be improved if a variable efficiency for milk protein production is used. In the DVE-system the observed efficiency decreased with increasing protein to energy ratio and milk production level.

During the development of the DVE-system, it became apparent that more information was needed to enable a more detailed description of N digestion, including the fermentation of organic matter in the rumen and digestion of protein in the intestine of forages and the effect of fermentation and digestion on the amino acid supply to the animal. These subjects were studied in experiments reported in Chapter 4, 5 and 6.

Protein in fresh grass and grass silage is highly fermentable in the rumen and can result in large N losses from the rumen. The fermentability is influenced by the N-fertilization level, season and maturity of the sward. The recent trend towards a reduction of N-fertilization enables the introduction of clover in the sward. The effect of season and maturity on rumen degradation of organic matter and protein and intestinal digestibility of protein was compared between intensively N-fertilized grass sward and moderately N-fertilized grass and clover from a mixed sward (Chapter 4). From May to September, eight consecutive cuts of both swards were made, at a late and early stage of maturity. Samples were incubated in the rumen and intestine of dairy cows fitted with rumen and duodenal cannulae, using *in sacco* methods. Clover had a higher content of effectively rumen fermentable organic matter and protein than grass, due to higher soluble fractions, lower undegradable fractions and higher degradation rates. During the season the fermentable organic matter and protein of grass and clover decreased, due to a decrease in soluble fractions and increase in undegradable fractions. Effects of season on degradation rates varied between grass and clover. A more mature sward had a lower fermentable organic matter and protein than a young sward. Undegradable fractions were higher, soluble fractions and degradation rate were lower. The N-fertilization level had limited effects on rumen degradation and intestinal digestion of grasses. To prevent large N loss from the rumen, clover based diets should be supplemented with high fermentable energy sources. Because of the higher protein content, clover had a higher content of escape protein and intestinal digestible protein compared to grass.

In Chapter 5 the mobile nylon bag method to estimate the digestibility of escape protein of forages was evaluated and compared to other methods. Samples of grass, clover and grass silage were obtained from rumen nylon bag experiments. In two experiments rumen

and intestinal disappearance of protein from nylon bags was measured in dairy cows fitted with rumen and duodenal cannulae. Total tract indigestible protein measured with this method was compared to the indigestible protein predicted by *in vitro*, prolonged rumen incubation (14 d) and two stage rumen incubation (12 h followed by 14 d) methods. Ruminal disappearance of protein from nylon bags was higher for grass silage than for grass and clover, and intestinal protein disappearance was higher for clover than for grass and grass silage. Within a forage, a lower ruminal disappearance was compensated by a higher intestinal disappearance, resulting in a small variation in total tract indigestible protein. The average total tract indigestible protein measured with the mobile nylon bag method was lower than estimated by the *in vitro,* prolonged rumen incubation and two stage rumen incubation methods. All methods ranked forages similarly, resulting in high correlation coefficients among methods. Differences between methods can be due to differences in enzyme activity and concentration, hindgut fermentation, washing procedure and grinding of samples. Total tract indigestible protein of grass, grass silage and clover was related to protein content and harvest date, dry matter and acid-detergent-insoluble-N content, and dry matter and protein contents, respectively.

In Chapter 6 the change of amino acid content and profile in protein of feedstuffs during digestion was studied. Using the nylon bag methods, six concentrate ingredients, two roughages and one concentrate were subjected to washing, rumen and intestinal incubation. Additivity of disappearances was studied by comparing the observed values for the concentrate with the calculated values based on concentrate ingredients. The disappearance of amino acid N and non protein N after washing, rumen and intestinal incubation varied between feedstuffs. For beetpulp, grass silage and maize silage, washing showed a considerable effect on the amino acid profile. Rumen incubation had only a small effect on the amino acid profile of the residue after washing. Amino acid profiles of rumen residues of beetpulp, grass silage and maize silage might be contaminated with microbial amino acids. Disappearance of amino acid N after intestinal incubation was higher than non protein N disappearance. Intestinal amino acid N disappearance was related to total N disappearance. Intestinal incubation showed a considerable effect on the amino acid profile for all feedstuffs. It was concluded that small intestinal digestible protein had a higher amino acid N in total N than the feedstuff and that this profile of concentrates could be calculated from that of the ingredients.

Information from Chapter 2 to Chapter 6 and from literature and unpublished experiments was used to develop and validate a model to predict amino acid N and non protein N flow in the animal and N excretion in milk, faeces and urine (Chapter 7). The model described digestion and metabolism in four compartments: rumen, small intestine, large intestine and metabolism. Because energy and protein interact at several levels in the animal, also fermentation and digestion of other compounds of organic matter than N were described. Fermentation and microbial growth in the rumen and large intestine was based on degradation characteristics measured with nylon bag incubations and fractional passage rates measured with markers. Small intestine digestion and efficiency factors in

the metabolism were described by coefficients.

The sensitivity analysis revealed that predicted N flows and excretions were very sensitive to the digestibility coefficients and endogenous contribution of N in the small intestine. Fractional passage rates only had a minor effect on N flows and excretions. The predicted N flow in the duodenum and ileum and N excretion with milk, faeces and urine were compared to experimental data. The N-flow model could predict total duodenal N flow, but underestimated the microbial contribution, probably due to inadequate description of the cell wall fermentation or underestimation of the fractional degradation rates. To obtain a close agreement between predicted and observed N flow in the ileum, negative digestibility coefficients for endogenous N were necessary. The modified N-flow model overestimated faecal N excretion and underestimated microbial N contribution, probably due to inadequate description of organic matter fermentation in the large intestine. Urinary N excretion was overestimated. The main and most variable source of urinary-N was N lost in the rumen. Milk N production could be accurately predicted due to a variable efficiency of milk protein production.

Chapter 8 is focused on the further development of protein evaluation. With the DVE-system as a starting point, current methods to estimate protein values and easy and cheap alternative methods to enable rapid screening of the protein value of feedstuffs in practise are discussed. The nylon bag method to estimate escape protein can be improved by further standardization of the methodology. A promising alternative is an *in vitro* method with a cocktail from Streptomyces griseus, but further development and standardization is required. Several methods to estimate intestinal digestibility of escape protein and fermentable organic matter are described. Some methods can not be regarded as practical alternative because they still require cannulated animals, while other methods need further development and validation before conclusions can be drawn.

To improve the estimation of N excretions in dairy cows protein evaluation systems should be further developed towards dynamic models that describe N digestion and metabolism in more detail. With the N-flow model as example, it was shown that such an approach would result in different protein values for feedstuffs high in protein compared to the DVE-system. Extension of protein evaluation towards individual amino acid evaluation requires additional information on amino acid profiles of escape, microbial and endogenous protein. Requirements should be established by relating calculated amino acid supply to obtained milk protein production.

Finally, based on the estimations of the N-flow model, possibilities to reduce N losses in dairy cows are summarized. Rumen N loss can be minimized by optimising the ratio between fermentable protein and organic matter and increasing the efficiency of microbial protein synthesis. Nitrogen loss due to inefficient use of amino acids for milk production can be reduced by optimizing the ratio between energy and protein available for milk production and balancing the amino acid supply. The excretion of endogenous N in gastrointestinal tract resulted in substantial losses of N in faeces and urine. More research is needed to obtain information on the extend and factors influencing these losses.

SAMENVATTING

Gedurende de laatste decennia heeft de melkveehouderij in Nederland zich ontwikkeld tot een intensief produktie systeem, waarin hoge melkprodukties per koe gehaald worden. Dergelijke systemen kunnen een belasting vormen voor het milieu. De Nederlandse melkveehouderij wordt verantwoordelijk gehouden voor een belangrijk deel van de N vervuiling van lucht en grondwater. Verschillende methoden om N verliezen op bedrijfsniveau te beperken zijn bestudeerd, waaronder de mogelijkheden via de voeding. Gemiddeld wordt 80 tot 85 procent van de N opname van een melkkoe uitgescheiden met mest en urine. Deze verliezen kunnen worden teruggedrongen wanneer het aanbod van eiwit (Nx6,25) zowel kwantitatief als kwalitatief wordt afgestemd op de behoefte van het dier. Echter, het eiwitwaarderingssyteem dat tot voor kort in Nederland werd gebruikt (het VRE-systeem), heeft beperkte mogelijkheden om het effect van voeding op N verliezen te voorspellen, voornamelijk omdat de eiwitafbraak en microbiële eiwitsynthese in de pens niet in beschouwing werden genomen. Daarom was het noodzakelijk om een nieuw eiwitwaarderingssysteem te ontwikkelen op basis van recent verkregen kennis van eiwitvertering en -metabolisme.

Het doel van dit proefschrift was om een model te ontwikkelen dat N uitscheiding in melk, mest en urine voorspeld. Als N vertering en metabolisme gedetailleerd beschreven worden, kunnen eveneens de oorzaken van onnodige N verliezen geïdentificeerd worden. De ontwikkeling van het model werd gelijktijdig uitgevoerd met de ontwikkeling en invoering van een nieuw eiwitwaarderingssysteem bedoeld voor praktisch gebruik in Nederland (het DVE-systeem).

De ontwikkeling van het model startte met het verzamelen en evalueren van informatie uit literatuur en eerder uitgevoerde proeven (Hoofdstuk 2 en 3).

In Hoofdstuk 2 wordt een overzicht gegeven van recente informatie over de eiwitafbraak in de pens en -vertering in de dunne darm. Er bestaan verschillende methoden om eiwitafbraak in de pens te bepalen. De toepasbaarheid van de *in vivo* methode wordt beperkt omdat het een indirecte methode is waarbij gebruik wordt gemaakt van dieren met een canule in het duodenum. *In vitro* methoden moeten verder ontwikkeld worden om goed vergelijkbare resultaten te krijgen met *in vivo* eiwitafbreekbaarheid. Wanneer een goed gestandaardiseerde procedure wordt gebruikt, is de *in sacco* methode momenteel de beste methode om eiwitafbraak in de pens te bepalen. Met deze methode kan eiwit onderverdeeld worden in een oplosbare fractie, een onverteerbare fractie en een onoplosbare, potentieel verteerbare fractie met bijbehorende afbraaksnelheid. Resultaten van proeven van het ID-DLO (voorheen IVVO-DLO) werden op deze manier gepresenteerd. In combinatie met een veronderstelde passagesnelheid kan uit deze afbraakkenmerken een bestendige fractie berekend worden. Gegevens van eiwitbestendigheid van krachtvoergrondstoffen werden verzameld uit de literatuur en gecorrigeerd voor verschillen tussen laboratoria. Er was een grote variatie tussen grondstoffen in eiwitbestendigheid. De variatie in afbraakkenmerken van ruwvoeders kon gerelateerd worden aan de samenstelling en oogstdatum van het ruwvoer. Vertering van bestendig eiwit kan worden gemeten met *in vivo* en *in sacco* methoden. Uit literatuur

gegevens bleek dat de mobiele nylon zakjes methode resulteerde in een hogere verteerbaarheid dan de infusie methode. Eiwitafbraak en -vertering kunnen beïnvloed worden door verandering van groeiomstandigheden (ruwvoeders), behandeling (fysisch, chemisch) en voedingsstrategieën.

In verschillende landen zijn nieuwe eiwitwaarderingssystemen ontwikkeld en ingevoerd om het VRE-systeem te vervangen. Hoewel ze gebaseerd werden op dezelfde uitgangsgegevens, was er een groot verschil tussen systemen in optimale eiwitgehalte en bestendig eiwit en de voorspellingsnauwkeurigheid van melkeiwitproduktie. In Hoofdstuk 3 zijn verschillende eiwitwaarderingssytemen (RE-, VRE-, PDI-, AAT-, AP-, MP, AAS- en DVE-systeem) gevalideerd met behulp van gegevens van 15 voederproeven met melkkoeien. Alleen behandelingen die in tenminste 1 systeem een tekort aan eiwit hadden, werden hiervoor geselecteerd. Het gemiddelde produktieniveau van deze behandelingen was 989 g melkeiwit per dier per dag. De melkeiwitproduktie werd voor ieder systeem geschat uit het aanbod en behoefte van de dieren. De schattingsfout was het kleinst voor het DVE-systeem, en nam toe in de volgorde: RE-, PDI-, VRE-, AP-, AAS-, AAT- en MP-systeem. De voorspellingen kunnen mogelijk worden verbeterd als een variabele efficiëntie van melkeiwitproduktie gebruikt wordt. Voor het DVE-systeem bleek deze efficiëntie te dalen met een toenemende melkproduktieniveau en eiwit/energie verhouding in het voer.

Tijdens de ontwikkeling en invoering van het DVE-systeem bleek dat er een aantal hiaten waren in de kennis om de N vertering en metabolisme in meer detail te kunnen beschrijven. Dit betrof onder andere de fermentatie en vertering van eiwit in ruwvoeders, en de invloed van fermentatie en vertering op aminozuursamenstelling en aminozuurgehalte van voereiwit. In Hoofdstuk 4, 5 en 6 worden de resultaten van proeven beschreven die gedaan werden om hier meer informatie over te krijgen.

Eiwit in vers gras en grassilage is goed fermenteerbaar en kan leiden tot grote N verliezen in de pens. De mate van fermentatie wordt beïnvloed door de N-bemesting, het seizoen en de ouderdom van het gewas. De huidige trend tot verlaging van N-bemesting maakt introductie van klaver in de weide mogelijk. In Hoofdstuk 4 werd de invloed van seizoen en ouderdom op fermentatie van organische stof en eiwit en darmverteerbaarheid van eiwit vergeleken tussen gras van een intensief N-bemest perceel met gras en klaver van een matig N-bemest perceel. Van mei tot september werden acht sneden gemaakt van beide percelen, op een laat en vroeg stadium van ouderdom. De monsters werden in nylon zakjes in de pens en darm van melkkoeien voorzien van canules geïncubeerd. Klaver had een hoger gehalte aan effectief gefermenteerde organische stof en eiwit dan gras, door hogere oplosbare fracties en afbraaksnelheden, en lagere onverteerbare fracties. Gedurende het seizoen nam de fermenteerbare organische stof en eiwit af als gevolg van een afname in de oplosbare en toename in de onverteerbare fractie. Seizoenseffecten op de afbraaksnelheid waren verschillend voor gras en klaver. Een ouder gewas resulteerde in lagere fermenteerbare organische stof en eiwit dan een jong gewas. De onverteerbare fracties waren hoger en de oplosbare fracties lager. Er was voor alle

gemeten kenmerken geen verschil tussen gras van het hoog en matig N-bemest perceel. Om hogere N verliezen in de pens te voorkomen is het gewenst rantsoenen met klaver aan te vullen met goed fermenteerbare energiebronnen. Vanwege het hogere eiwitgehalte had klaver een hogere bestendig eiwit- en darmverteerbaar eiwitgehalte vergeleken met gras.

In Hoofdstuk 5 wordt de bepaling van de darmverteerbaarheid van eiwit in ruwvoeders met behulp van de mobiele nylon zakjes methode geëvalueerd en vergeleken met andere methoden. Monsters van vers gras, klaver en grassilage waren beschikbaar uit eerder uitgevoerde experimenten. In twee proeven werd de verdwijning van eiwit uit nylon zakjes in de pens en darm van melkkoeien gemeten. De onverteerbare eiwit fractie in het maagdarmkanaal bepaald met deze methode werd vergeleken met de onverteerbare eiwit fractie volgens een *in vitro*-, langdurige pensincubatie- (14 dagen) en twee-traps-pensincubatiemethode (12 uur gevolgd door 14 dagen). De verdwijning van eiwit uit nylon zakjes in de pens was hoger voor grassilage dan voor vers gras en klaver. De verdwijning in de darm was hoger voor klaver dan voor grassilage en vers gras. Een lagere verdwijning in de pens werd binnen hetzelfde voedermiddel gecompenseerd door een hogere verdwijning in de darm, resulterend in beperkte variatie in de onverteerbare eiwit fractie over het totale maagdarmkanaal. De gemiddelde onverteerbare eiwit fractie gemeten met de mobiele nylon zakjes was lager dan bepaald volgens de *in vitro*-langdurige pensincubatie- en twee-traps-pensincubatiemethoden. De ruwvoeders werden wel op vergelijkbare wijze gerangschikt voor iedere methode, resulterend in hoge correlatiecoëfficiënten tussen methoden. Verschillen tussen methoden kunnen veroorzaakt zijn door verschil in enzym-activiteit en -concentratie, dikke darm fermentatie, de wasprocedure en het malen van monsters. De onverteerbare eiwit fractie van gras, grassilage en klaver was gerelateerd aan respectievelijk het eiwitgehalte en oogstdatum, het droge stof- en acid-detergent-insoluble-N-gehalte en aan het droge stof- en eiwitgehalte.

In Hoofdstuk 6 is de invloed van vertering op het aminozuurgehalte en -patroon van voereiwit onderzocht. Met behulp van de nylon zakjes methode werden zes krachtvoer grondstoffen, twee ruwvoeders en een krachtvoer onderworpen aan wassen, pens- en darmincubatie. De additiviteit van de metingen werd nagegaan door de verdwijning van aminozuren in het krachtvoer, berekend op basis van de grondstoffen, te vergelijken met de bepaalde verdwijning. De verdwijning van aminozuur-N na wassen, pens- en darmincubatie varieerde tussen voedermiddelen. Bij bietenpulp, grassilage en maissilage had wassen een aanzienlijk effect op het aminozuurpatroon. Pensincubatie had weinig invloed op het aminozuurpatroon van het eiwit na wassen. De aminozuurprofielen van pensresiduen van bietenpulp, grassilage en maissilage zouden besmet kunnen zijn met microbiële aminozuren. De verdwijning van aminozuur-N in de darm was hoger dan de verdwijning van niet-eiwit-N. De verdwijning in de darm van aminozuur-N was gerelateerd aan die van totaal N. Incubatie in de darm had een groot effect op het aminozuurpatroon bij alle voedermiddelen. Geconcludeerd werd dat darmverteerbaar voereiwit een hoger gehalte aan aminozuur-N in totale N heeft dan eiwit in het

voedermiddel, en dat deze gehaltes voor krachtvoer goed op basis van de grondstoffen berekend kunnen worden.

Informatie uit Hoofdstukken 2 tot 6 en uit de literatuur en niet gepubliceerde proeven werd gebruikt om een model te ontwikkelen dat de aminozuur-N en niet-eiwit-N stroom in het dier en N uitscheiding met de melk, mest en urine beschrijft (Hoofdstuk 7). Het model beschrijft de vertering en het metabolisme in vier compartimenten: de pens, dunne darm, dikke darm en het metabolisme. Omdat eiwit en energie op verschillende niveaus in het dier een wisselwerking hebben, werden ook de fermentatie en vertering van alle onderdelen van de totale OS beschreven. De fermentatie en microbiële groei in de pens en dikke darm was gebaseerd op afbraakkenmerken gemeten met nylon zakjes en passagesnelheden gemeten met merkstoffen. De vertering in de dunne darm en de efficiëntiefactoren in het metabolisme werden beschreven als coëfficiënten.

Uit de gevoeligheidsanalyse kwam naar voren dat de voorspelde N stroom en uitscheiding sterk beïnvloed werd door de verteringscoëfficiënten in de dunne darm en de endogene bijdrage van N in de dunne darm. De passagesnelheid had hier minder invloed op. De voorspelde N stroom in het duodenum en ileum en N uitscheiding met melk, mest en urine werd vergeleken met experimentele gegevens. Het model kon de N stroom door het duodenum voorspellen, maar onderschatte het microbiële aandeel, mogelijk als gevolg van een onjuiste beschrijving van de celwandfermentatie of onderschatting van de afbraaksnelheden. Om een goede overeenstemming tussen voorspelde en gemeten N stroom in het ileum te krijgen was het noodzakelijk om negatieve verteringscoëfficiënten voor endogeen N in het model op te nemen. Nader onderzoek naar de bijdrage en vertering van endogeen N is noodzakelijk. Het model liet een overschatting van de N uitscheiding in de mest zien, met een onderschatting van het microbiële aandeel, mogelijk als gevolg van een onjuiste beschrijving van de fermentatie in de dikke darm. Ook de N uitscheiding in de urine werd overschat. De grootste en meest variabele bron van urine-N was het verlies van N in de pens. Uitscheiding van N in de melk kon goed voorspeld worden als gevolg van de variabele efficiëntie van melkeiwitsynthese.

Hoofdstuk 8 behandelt de verdere ontwikkeling van eiwitwaardering. Met het DVE-systeem als uitgangspunt worden de huidige methoden en mogelijke eenvoudige en praktisch toepasbare alternatieven voor de schatting van de eiwitwaarde van voedermiddelen besproken. De nylon zakjes methode kan worden verbeterd door verdere standaardisering. Een veelbelovend alternatief is de *in vitro* methode met een enzymmengsel van Streptomyces griseus, maar verdere ontwikkeling en validatie moeten nog worden uitgevoerd. Verschillende methoden om de verteerbaarheid in de darm van bestendig eiwit en de fermenteerbare organische stof worden besproken. Een aantal van deze methoden gelden niet als alternatief omdat nog steeds gecanuleerde dieren nodig zijn, terwijl andere nog verder ontwikkeld en gevalideerd moeten worden, voordat conclusies over de bruikbaarheid in de praktijk kunnen worden getrokken.

Om de schatting van N uitscheiding door melkkoeien te verbeteren dienen de praktisch toepasbare eiwitwaarderingssystemen verder te worden ontwikkeld richting dynamische

modellen die de N vertering en metabolisme in meer detail beschrijven. Met het N-stroom model als voorbeeld, bleek dat een dergelijke benadering resulteerde in hogere eiwitwaarden voor voedermiddelen met een hoog eiwitgehalte vergeleken met het DVE-systeem. Voor de uitbreiding van eiwitwaardering richting individuele aminozuur waardering zijn meer gegevens over het aminozuurpatroon van bestendig voer-, microbiëel- en endogeen eiwit nodig. De behoeften zouden moeten worden afgeleid door de berekende aminozuur opname te relateren aan de behaalde melkproduktie.

Tot slot is een samenvatting gegeven van de mogelijkheden om N verliezen bij melkvee terug te dringen. De verliezen in de pens kunnen worden verminderd door optimalisatie van de eiwit en organische stof fermentatie in de pens en toename van de microbiële efficiëntie. N verliezen als gevolg van inefficiënte benutting van aminozuren voor melkproduktie kunnen teruggedrongen worden door optimalisatie van de eiwit/energie verhouding in het rantsoen en afstemmen van het aanbod van individuele aminozuren op de behoefte. De uitscheiding van endogeen N in het maagdarmkanaal resulteert in omvangrijke verliezen van N in de mest en met de urine. Er is echter meer onderzoek nodig om de omvang van deze verliezen en factoren die er invloed op uitoefenen in kaart te brengen.

CURRICULUM VITAE

Werenfridus Maria van Straalen werd geboren op 3 maart 1964 te Berkhout. In 1982 behaalde hij het Atheneum-B diploma aan de Scholengemeenschap Werenfridus gevestigd in Hoorn en begon hij de studie aan de toenmalige Landbouwhogeschool in Wageningen in de richting Zoötechniek. In 1988 studeerde hij af aan de Landbouwuniversiteit Wageningen, met als hoofdvak Veehouderij en als bijvakken Veevoeding en Dierfysiologie. Direct daarna kwam hij in dienst van de Landbouwuniversiteit Wageningen als Assistent In Opleiding en werd gedetacheerd bij het toenmalige Instituut voor Veevoedingsonderzoek in Lelystad, waar het grootste deel van het in dit proefschrift beschreven onderzoek werd verricht. Ten bate van dit onderzoek werd een periode van 3 maanden doorgebracht bij het Station de Recherche sur la Vache Laitière van het INRA in Rennes (Frankrijk). In november 1992 trad hij als onderzoeker in dienst van het CLO-Instituut voor de Veevoeding "De Schothorst" te Lelystad, met als vakgebied de voeding van de herkauwer. Daar werd het onderzoek beschreven in dit proefschrift voortgezet en afgerond.

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