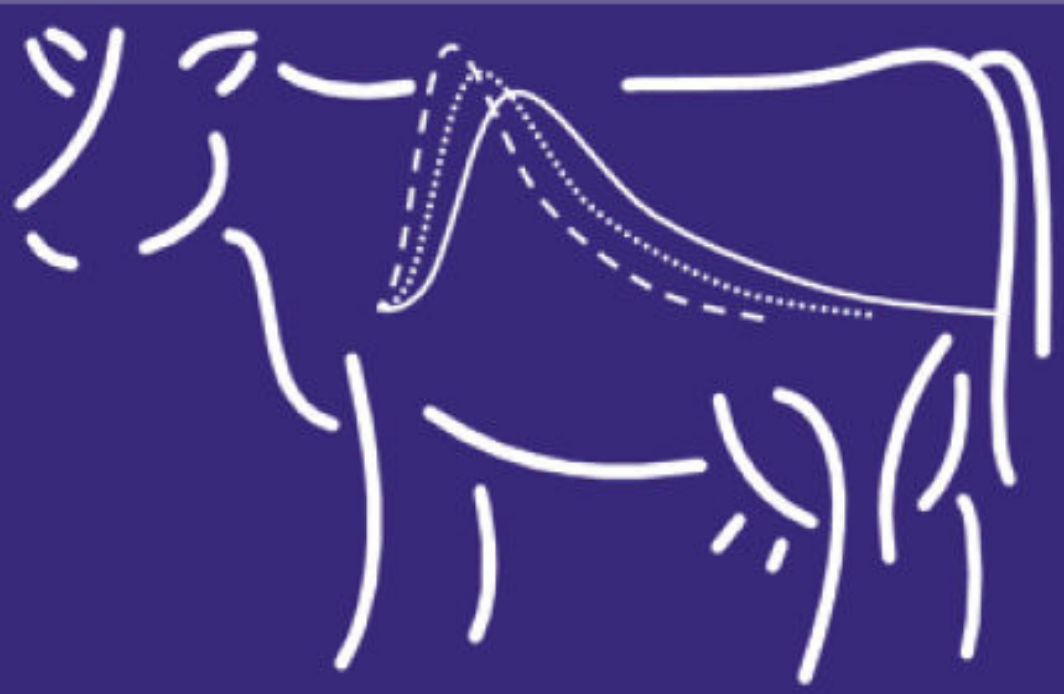
^{13}C-labelled feed components

Passage of ^{13}C-labelled feed components through the digestive tract of dairy cows



Wilbert F. Pellikaan

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Promotor:	Prof. dr. ir. S. Tamminga Hoogleraar in de Diervoeding, Wageningen Universiteit	
Co-promotoren:	Dr. ir. J. Dijkstra Universitair docent, leerstoelgroep Diervoeding, Wageningen Universiteit	
	Dr. ir. H. Boer Universitair docent, leerstoelgroep Diervoeding, Wageningen Universiteit	
Promotiecommissie:	Prof. dr. ir. B. Kemp	Wageningen Universiteit
	Prof. dr. D. Poppi	University of Queensland, Australia
	Dr. ir. W.M. Van Straalen	CLO Instituut “De Schothorst”, Lelystad
	Dr. E. Kebreab	University of Guelph, Canada

Dit onderzoek is uitgevoerd binnen de onderzoekschool
Wageningen Institute of Animal Sciences

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Wilbert F. Pellikaan

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speelman,
in het openbaar te verdedigen
op dinsdag 14 september 2004
des namiddags te twee uur in de Aula.

Pellikaan, W.F., 2004. Passage of ^{13}C-labelled feed components through the digestive tract of dairy cows

Ph.D. Thesis, Wageningen Institute of Animal Sciences, Animal Nutrition Group, Wageningen University, PO Box 338, 6700 AH Wageningen, The Netherlands

With summaries in English and Dutch

ISBN: 90-8504-075-2

ABSTRACT

The main aim of this thesis was to acquire knowledge on the feed specific fractional passage rates of feedstuffs and feed components through different compartments of the gastro-intestinal tract of dairy cows. Fractional passage rates form, next to fractional degradation rates, the determining factors to quantify the escape part of feed components from the rumen. Current feed evaluation systems use feed specific fractional degradation rates, but adopt fixed fractional passage rates. It is increasingly realised that also passage rates vary, amongst others with the level of feed intake, diet quality and diet composition. Besides, international developments in feed evaluation move towards so-called "nutrient based" feed evaluation systems that are more dynamic in nature. Knowledge on feed specific fractional passage rates is essential to further develop such dynamic systems. To meet our main aim, several animal trials were conducted in which the passage behaviour of feed particles originating from grass and grass silages were studied and compared with the traditionally used external markers Cr-NDF and Co-EDTA, using rumen and ileum cannulated animals. The roughages were labelled with the stable isotope of carbon (^{13}C), thus allowing to follow the passage behaviour of the dry matter, the cell wall and the non cell wall fractions. In all cases, ^{13}C gave slower ruminal passage rates compared to the external markers, and with respect to the labelled fractions the ^{13}C-labelled cell wall fractions gave the slowest fractional passage rates. A reduction in the level of feed intake of 40% gave decreased marker passage rates with the highest impact on the rumen residence times for the cell wall fractions. The effect of roughage quality on ruminal marker passage was more pronounced for the internal markers compared to the external markers. In combination with *in situ* degradation characteristics our data indicates that ^{13}C seems to be a useful tool to quantify the relations between passage and degradation. Based on our findings it was concluded that the fixed fractional passage rates currently adopted in the Dutch protein evaluation system overestimate the *in vivo* situation. Besides, our data suggests the possibilities to introduce feed component specific fractional passage rates as a function of animal and dietary characteristics.

Keywords: fractional passage rate, grass, silage, ^{13}C-isotope, internal markers, dairy cows

PREFACE

In February ’97 when I was doing parttime work at the Physiology group, I coincidently met Germ Hof. I was keen on getting scientific work experience, and he could use a pair of extra hands at the Ossekampen where Wouter van Gestel was about to start a major experiment with dairy cows. During one of Germ’s visits to the barn we had a talk about a possible PhD position that would include both ruminant and agronomical aspects; ánd stable isotopes were involved as well. So, exciting high-tech stuff and I was as happy as can be, when after a few months I heard I had the job. That was seven years ago. Now at this moment, the book is near completion and it’s time for some final contemplation about these years, the work and about all the people who in various ways contributed to it, and made it possible that I could finalise it in the form of this thesis.

First of all I would like to acknowledge my team of promotor and co-promotors. Seerp, I sincerely appreciate your input in my project. I could not have wished for a better tutor/ mentor to ‘introduce’ me into the science of ruminant nutrition. Jan, your contributions have been most valuble. It is very good, to know that a sharp eye is looking over one’s shoulder.
Huug, I’m happy you are seated next to Jan on stage. The time we spend in the lab and field, calculating and experimenting to set up the labelling procedures I appreciate very much.
A major part of my time was spend at the Ossekampen, doing labelling experiments during the summer period and running animal experiments in the barn during the winterperiod. Regarding the labelling trials, my house mates played an essential role in taking shifts and join me in the night to move the assimilation cages. A big ‘thank you’ to all for helping out. From a labor technical point of view, I combined my work in the barn with the experimental work from one of my collegues, Wouter. Wouter, although your way of working was slightly different from mine I very much enjoyed our combined experimental time at the farm, and especially your share of the cakes you brought to the farm.
To bring the experimentes to a successful ending a skillfull staff is essential. Arie, Leen, Ilona, Ronald, Wian, Wim, and Joop, thanks for all the assistance. Many students have been participating in the project: Adriaan, Hans, Guido, Annemiek, Deelip, Bart, Marcel, Robert, Joana, Frank, Kees, Sjaak and Shaula; your input and efforts are highly appreciated. After collecting the samples and storing them in the freezer, you’re not even halfway. Toos, you always managed to squeeze some of my samples in the queue, so I could continu labelling, ensiling, etc. But also your contribution in the technical aspects on ^{13}C I regard as very valuble; Thank you! Truus, Jane-Martine, Meijke and Marjan, many thanks for all the work you did in my thousands of samples. Marjan, the (I don’t know how many you did) NDR analyses was an enormous job, an excellent job with nice results. Dick, your input in the VFA analyses is highly appreciated. The last but not least in this line is my former office mate Jan Lenaers. Jan, you did a lot of grinding and sample preparation for ^{13}C-analyses, which is not the most exiting work, but to me it was an essential step. Many thanks for that.

Talking about former office mates; Barbara, I really enjoyed sharing the office with you, the more scientific and less scientific talks we've had. Your listening ear and suggestions formed a good support during the last 'writing' part of the thesis. I wish you and JanKees and Pieter all the best in Australia and hope to see you in time somewhere.
Since the time I came to Animal Nutrition many people with a wide variety of nationalities came and went. This international character of our group makes it a rather special group to be part of. Therefore, thank you all for your good company and hopefully we keep in touch.
My fellow PhD collegues, also many there were. I started in a time when Menno, Jos, Jacob, Pablo, Carina, Harmen, Witek and William formed the hardcore PhD-crew, who showed me how 'serious' science could be. Special thoughts go out to Sandra and Martin. Martin, ze waren het allemaal waard! (Rijnen, 2003). Sandra, you are a great person. I really look back in fond memory to the time both of you were here at ANU. To the fellow PhD-ers I can say that it's great to be in your midst.
The person I started this preface with, and who actually introduced me to Animal Nutrition was Germ. Germ was a member of my supervising team and especially during the years of the experimentation, I had a lot of contact/ discussions with him. Germ seemed to be an endless resource of knowledge on animal experiments, and what to do when things/ animals didn't work as they should. Sadly, Germ passed on shortly after his retirement, a great loss to his family and our group. I think back in fond memory of the times I worked with him, and think I'm a lucky person in that I had the opportunity to work under his supervision.

Heit en Mem, jullie hebben heel wat verhalen over de koeien en het werk aan moeten horen de afgelopen jaren. Bedankt voor jullie belangstelling en steun. Het boekje is klaar, tijd voor het volgende hoofdstuk.
Joana, sol de minha vida! I think I'm a rich person having you next to my side with our two girls. You have been a great support, tu es demais, obrigado. This book is also yours.

Wilbert

TABLE OF CONTENTS

Chapter 1

General Introduction

General Introduction

Fractional Passage in Relation to Feed Evaluation

During the last decades, feed evaluation in ruminants has been subject to major changes. In 1977 the Starch Equivalent (SE) system was replaced by the VEM system. In 1991 a new protein evaluation system (DVE-system) replaced the digestible crude protein (DCP) system in the Netherlands. Shortcomings of the DCP-(and in fact also the VEM) system are that they do not account for microbial conversions (degradation and synthesis) in the forestomachs, making it impossible to accurately quantify the relation between diet composition and the supply of nutrients to the animal (Van Straalen, 1995; Tamminga 1975).

Within the DVE-system protein supply to individual animals is described as the sum of a microbial component (DVME), a bypass feed protein component (DVBE), a correction factor for endogenous losses (DVMFE) and the digestibility of these components in the small intestine. The DVME is considered to depend directly on the amount of organic matter fermented (FOM) in the forestomachs. An important component of FOM is starch and thus DVME partly depends on the amount of starch escaping from rumen fermentation. The rumen escape part of feed protein and starch are feed specific and depend on the balance between the fractional rates of degradation and passage. Degradation rates are obtained by use of *in sacco* experiments. To quantify the escape part of feed components, fixed fractional passage rates have been adopted; 0.045/h for protein in roughages, 0.060/h for protein in concentrates (CVB, 1991; Tamminga *et al.*, 1994; Vérité *et al.*, 1987) and 0.050/h for escape starch (Tamminga *et al.*, 1990). It is increasingly realised that passage rates are also feed specific and vary with diet quality (Bruining and Bosch, 1991; Rinne *et al.*, 1997), diet composition and level of feed intake (Tamminga *et al.*, 1989[a]; Colucci *et al.*, 1990).

International developments in feed evaluation move towards the integration of energy and protein evaluation in systems of a more dynamic nature, the so-called “nutrient based” feed evaluation systems (Baldwin, 1995; AFRC, 1998; Tamminga *et al.*, 2000). In such dynamic systems, fractional passage rates form important factors as they determine the site of degradation and the nature of nutrient supply, for instance the efficiency of microbial protein synthesis (Dijkstra and France, 1996). Hence, for the further development of nutrient based feed evaluation systems, knowledge about feed specific retention times (the reciprocal of the fractional passage rates) is essential information, which up-to this moment is still lacking.

Fractional Passage in Relation to Digesta Markers

Conventional studies on passage dynamics of feed particles often make use of external (e.g., Cr-NDF) or inert internal markers (e.g., acid-insoluble ash, indigestible fibre fractions).

For external markers it is assumed that they follow and represent the passage behaviour of the particles ingested with the diet. However, it can be hypothesised that such markers behave different than feed particles and do not accurately describe the actual situation (Tamminga *et el.*, 1989[b]). The inert internal markers referred to earlier, circumvent these problems as they form intrinsic components of the feed particles. However, in this case the markers are not unique and cannot be distinguished from the bulk material. Hence, information on passage dynamics from the rumen can only be obtained from frequent evacuations of the rumen content. This is a very time consuming and labour intensive procedure. Besides, legislative demands to restrict the use of experimental animals make this technique increasingly unfavourable. Therefore, it is desirable to use markers that evade the earlier mentioned limitations, and that are representative for the *in vivo* situation. Stable isotopes (^{13}C, ^{15}N) appear to be suitable internal markers to meet these conditions (Huhtanen and Hristov, 2001; Südekum *et al.*, 1995; Boutton, 1991). In the work as outlined in this thesis, ^{13}C was used as an internal marker by incorporating this isotope into the cell wall and non cell wall fractions of herbage growing under field conditions (Thompson, 1996; Svejcar *et al.*, 1993). These artificially labelled materials were then used to assess feed and feed component specific fractional passage rates through different sections of the gastro-intestinal tract of dairy cows.

Fractional Passage in Relation to Compartmentalisation

Many studies have been conducted to assess post rumen digesta passage, but none of these efforts are reflected in the feed evaluation systems currently in use. Fore instance, in the Dutch DVE-system a fixed digestibility coefficient (0.85) has been adopted for the microbial protein that enters the small intestine (Tamminga *et al.*, 1994), and variable coefficients derived from mobile nylon bag experiments are used for the bypass feed protein (Van Straalen and Tamminga, 1990). For the development of future nutrient based feed evaluation systems knowledge on residence times in the post rumen compartments is essential in order to predict the amount of digestion end-products formed in those compartments (AFRC, 1998). Also in mechanistic modeling of the ruminant gastro-intestinal tract, little attention has been given to develop models describing the post rumen compartments (Gerrits and Dijkstra, 2000). In models describing the 'whole-animal' nutrient utilisation (Rodrigues *et al.*, 2002; Dijkstra *et al.*, 1996), the post rumen supply of nutrients was assigned to four pools (VFA's, glucose, LCFA's, amino acids) that are proportional to the model estimates on rumen fermentation. Mills *et al*. (1999) presented a conceptual framework for a mechanistic model describing post rumen starch digestion. In addition, Mills *et al.* (2001) extended the rumen model of Dijkstra *et al.* (1992) to represent large intestinal fermentation, and again commented on the extremely limited availability of quantitative data regarding passage of digesta post-ruminally. Factors influencing the passage of digesta through the post rumen compartment are differences in particle density, the physical size of starch containing particles and the differences between liquid and solid

fractions (Kaske and Engelhardt, 1990; Owens *et al.*, 1986; O'Connor *et al.*, 1984). Although Mills *et al.* (1999) identified factors other than fractional passage rates to be the significant contributors to post rumen starch digestion (e.g. enzyme, particle size and absorptive-related factors), they acknowledge the importance of particle size in relation to passage and degradation.

Hypotheses and Objectives

Based on the aforementioned considerations, in the summer of 1997 a project was initiated to study the passage behaviour of feed particles and feed components through different compartments of the gastro-intestinal tract of dairy cows. To acquire information on feed specific fractional passage rates of different feedstuffs and feed components, the stable isotope of carbon (^{13}C) was used as an internal marker. Four objectives were determined based on the hypotheses summarised below:

- The passage of the novel internal marker ^{13}C differs from the passage of traditionally used external markers.
- Different feeds and feed components show differences in passage behaviour.
- The passage of feeds and feed components are differently affected by factors like level of feed intake, diet composition and diet quality.
- The fractional rate of passage of feed particles from the rumen is largely related to the fractional rate of degradation in the rumen.

Therefore, the following objectives were stated:

- Determine the fractional passage rate constants of feed particles through different compartments of the gastro-intestinal tract, using an internal marker, and compare its passage behaviour with those estimated from the traditionally used external markers Cr-NDF and Co-EDTA.
- Quantify the differences in passage behaviour between the different feed components, e.g. the cell wall versus the non-cell wall fractions.
- Quantify the influence of the level of feed intake, roughage type and roughage quality on passage behaviour of the different feed components.
- Quantify the relation between the feed component specific fractional passage from the rumen and the fermentative degradation rates of these components.

Outline of the Thesis

To answer the hypotheses, first ^{13}C had to be incorporated into the herbage in order to create material that can be traced through the animals GI-tract. In two successive seasons,

grass was artificially enriched with ^{13}C under field conditions. A description of the procedure and some practical considerations for using ^{13}C as an internal marker are given in **Chapter 2**. Subsequently, these enriched materials were used in a series of animal experiments. In two successive experiments with three dairy cows, the differences in passage behaviour between the novel marker and that of traditional external markers as affected by the level of feed intake, were studied (**Chapter 3**). In an experiment with two animals the passage of feed particles originating from fresh grass was investigated (**Chapter 4**). In a double cross over experiment with dairy cows the effect of silage quality on passage characteristics was studied (**Chapter 5**).

As mentioned, integrated knowledge on residence times in the different compartments of the gastro-intestinal tract is essential for future developments in feed evaluation in ruminants. One of the factors of importance is the choice of model that describes the marker excretion pattern (Offer and Dixon, 2000). Different approaches in modelling procedures (e.g. stochastic versus deterministic) are available, and the type of model pre-determines in what way the tract of the animal is being compartmentalised, and to which compartments and segments of the intestinal tract a researcher will ascribe the rate constants. Differences in approaches, and the consequences for the estimation and interpretation of the rate constants generated by these models are dealt with in **Chapter 6**. In addition, the relations between compartmental residence times (and reciprocal fractional rate constants) and some animal and dietary characteristics are presented and discussed here.

One of the objectives in this project was to quantify the relation between passage and degradation. Diet components fed to the animals in the successive passage rate experiments, were evaluated for their *in situ* degradation characteristics in separate trials, but with animals kept under analogous dietary conditions. By combining the *in situ* data with the passage trials (Chapter 3, 4, 5) these relations could be studied and the results are presented and discussed in **Chapter 7**. Currently, within the DVE-system the fractional passage rates are still fixed at 0.045/h for roughages and 0.060/h in case of compound feeds. Adopting new values in this system may have a considerable impact on the estimations of the dietary protein and starch that will reach the intestine. Therefore in conclusion, the implications for the DVE-system and its fate are discussed, and scopes for the future are given (**Chapter 7**).

Literature Cited

AFRC. 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. AFRC Technical Committee on Responses to Nutrients. Report no. 11. Nutr. Abstr. Rev. (Ser. B) 68: 937-989.

Baldwin, R. L. 1995. Modeling ruminant digestion and metabolism. Chapman & Hall Ltd., London, UK.

Boutton, T.W. 1991. Uses and procedures for ^{13}C. *in*: Carbon isotope techniques. D.C. Coleman and B. Fry, eds. Academic Press Limited, London, UK:155-186.

Bruining, M. and M. W. Bosch. 1992. Ruminal passage rate as affected by CrNDF particle size. Anim. Feed Sci. Technol. 37(3-4):193-200.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, I. McMillan, and D. J. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 73(8):2143-2156.

CVB, Centraal Veevoeder Bureau. 1991. Eiwitwaardering voor herkauwers: het DVE-systeem. CVB-reeks nr. 7, ISSN nr. 0925-546X.

Dijkstra, J. and J. France. 1996. A comparative evaluation of models of whole rumen function. Ann. Zootechnie. 45:175-192.

Dijkstra, J., J. France, H. D. S. C. Neal, A. G. Assis, L. J. M. Aroeira, and O. F. de Campos. 1996. Simulation of digestion in cattle fed sugarcane: model development. J. Agric. Sci. 127(2):231-246.

Dijkstra, J., H.D.St.C. Neal, D.E. Beever and J. France. 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. J. Nutr. 122: 2239-2256.

Gerrits, W. J. J. and J. Dijkstra. 2000. Modelling growth and wool production in ruminants. Pages 343-361 *in* Feeding systems and feed evaluation models. M. K. Theodorou and J. France, eds. CABI Publishing, New York, USA.

Huhtanen, P. and A. N. Hristov. 2001. Estimating passage kinetics using fibre-bound N-15 as an internal marker. Anim. Feed Sci. Technol. 94(1-2):29-41.

Kaske, M. and W.V. Engelhardt. 1990. The effect of size and density on mean retention time of particles in the gastrointestinal tract of sheep. Brit. J. Nutr. 63:457-465.

Mills, J.A.N., J. Dijkstra, A. Bannink, S.B. Cammell, E. Kebreab and J. France. 2001. A mechanistic model of whole-tract digestion and methanogenesis in the lactating dairy cow: Model development, evaluation and application. J. Anim. Sci. 79: 1584-1597.

Mills, J. A. N., J. France, and J. Dijkstra. 1999. A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 2. postruminal starch digestion and small intestinal glucose absorption. J. Anim. Feed Sci. 8(4):451-481.

O'Connor, J. D., P. H. Robinson, C. J. Sniffen, and M. S. Allen. 1984. A Gastro-intestinal tract simulation model of digesta flow in ruminants. Can. Soc. Anim. Sci., occ. publ. 1.:102-122.

Offer, N. W. and J. Dixon. 2000. Factors affecting outflow rate from the reticulo-rumen. Nutr. Abstr. Rev. (Ser. B), Livestock Feeds and Feeding. 70(11):833-844.

Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits of starch digestion in the ruminant small intestine. J. Anim. Sci. 63(5):1634-1648.

Rodriques, S.I.F.C., J. Dijkstra, A.G. Assis, J. France and S. Tamminga. 2002. Evaluation of supplements to enhance nutrient supply and milk production of cattle fed diets based on elephant grass (*Pennisetum purpureum*) using a mathemetical model. Livest. Prod. Sci. 77:273-286.

Rinne, M., P. Huhtanen, and S. Jaakkola. 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol. 67(1):19-35.

Südekum, K. H., W. Ziggers, N. Roos, H. Sick, S. Tamminga, and M. Stangassinger. 1995. Estimating the passage of digesta in steers and wethers using the ratio of ^{13}C to ^{12}C and titanium(IV)-oxide. Isotopes Environ. Health Stud. 31:219-227.

Svejcar, T. J., M. B. Judkins, and T. W. Boutton. 1993. Technical note: Labeling of forages with ^{13}C for nutrition and metabolism studies. J. Anim. Sci. 71(2):1320-1325.

Tamminga, S. 1975. Some observations on protein digestion in the digestive tract of dairy cows. Miscellaneous Papers, Landbouwhogeschool Wageningen, Netherlands. 11(33-34.).

Tamminga, S., W. J. J. Gerrits, A. Bannink, J. Dijkstra, G. A. L. Meijer, and A. M. van Vuuren. 2000. Een opzet voor een nieuw, op nutriënten gebaseerd voederwaarderingssysteem voor herkauwers. 25ste studiedag voor Nederlandstalige voedingsonderzoekers, Wageningen:20-21.

Tamminga, S., P. H. Robinson, S. Meijs, and H. Boer. 1989[a]. Feed components as internal markers in digestion studies with dairy cows. Anim. Feed Sci. Technol. 27(1-2):49-57.

Tamminga, S., P. H. Robinson, M. Vogt, and H. Boer. 1989[b]. Rumen ingesta kinetics of cell wall components in dairy cows. Anim. Feed Sci. Technol. 25(1-2):89-98.

Tamminga, S., W. M. van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40(2):139-155.

Tamminga, S., A. M. van Vuuren, C. J. van der Koelen, R. S. Ketelaar, and P. L. van der Togt. 1990. Ruminal behaviour of structural carbohydrates, non-structural carbohydrates and crude protein from concentrate ingredients in dairy cows. Neth. J. Agric. Sci. 38(3B):513-526.

Thompson, R. B. 1996. Pulse-labelling a cover crop with ^{13}C to follow its decomposition in soil under field conditions. Plant Soil. 180(1):49-55.

Van Straalen, W. M. 1995. Modelling of nitrogen flow and excretion in dairy cows. Wageningen University, Wageningen.

Van Straalen, W. M. and S. Tamminga. 1990. Protein degradation of ruminant diets. Pages 55-72 *in* Feedstuff evaluation. J. Wiseman and D. J. A. Cole, eds. Butterworths, Guildford, UK.

Vérité, R., B. Michalet-Doreau, P. Chapoutot, J. L. Peyraud, and C. Poncet. 1987. "Révision du systeme des protéines digestibles dans líntestin (P.D.I.)". Bulletin Technique C.R.Z.V. Theix, INRA. 70:19-34.

Chapter 2

^{13}C-Enrichment of Cell Wall Fractions of Rye Grass (*Lolium Perenne* L.) and Implications for Its Use as Digesta Marker

W.F. Pellikaan[*], H. Boer[*], J. Dijkstra[*], G. Hof[*,1], S.C.W. Lammers-Wienhoven[†], and S. Tamminga[*]

[*]Animal Nutrition Group and [†]Human and Animal Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands

Submitted to Journal of Animal Science

[1] in memory of G. Hof

^{13}C-Enrichment of Cell Wall Fractions of Rye Grass (*Lolium Perenne* L.) and Implications for Its Use as Digesta Marker

W.F. Pellikaan, H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven, and S. Tamminga

Abstract

In order to obtain internal markers to study feed-specific passage behavior through the gastro intestinal tract of dairy cows, four labelling experiments were conducted where grass was enriched with the stable isotope of carbon, ^{13}C. In two successive years, small uniform and monospecific stands of rye grass (*Lolium perenne* L.) were selected in pastures and enriched with ^{13}C. In one case (Exp.2) the labelled material was kept as fresh grass, in the other cases (Exp.1, 3, 4) the enriched material was ensiled. Total C content (TC) and isotopic ratios (At%^{13}C and δ^{13}C in ‰ vs. Pee Dee Belemnite) were determined in the whole plant tissue (DM), neutral detergent residue (NDR), acid detergent residue (ADR) and the residue of acid detergent lignin (ADLR). ^{13}C-recovery in the DM was quantified to get insight in the efficiency and accuracy of our labelling procedure. Whole plant isotopic enrichments in the successive Exp. (1 to 4) were 627.1, 566.3, 786.6 and 496.7‰ vs. PDB. A multicompartmental model was used to assess the quantity of produced internal marker material in relation to the amounts required to enrich rumen pools when conducting animal experiments. In all cases adequate amounts of marker material were available to carry out the desired animal experiments. Distribution of ^{13}C over the different cell wall fractions, hemicellulose (628.4‰), cellulose (595.6‰) and lignin (389.2‰) were derived from the enrichment results in the NDR, ADR and ADLR. No statistical differences could be established between the levels of enrichment in the hemicellulose, cellulose and whole plant tissues. In all cases the ADL fraction had a significant ($P \leq 0.006$) lower level of enrichment. On the basis of these results it was concluded that under our experimental field conditions, rye grass discriminates against ^{13}C regarding the allocation of ^{13}C to the ADL fraction. This is probably primarily related to fractionation processes against ^{13}C that occur during the formation of biosynthetic precursors of lignin, phenylalanine and tyrosine. However, calculations show that changes in ADL/ NDF-ratio resulting from degradation are probably of negligible consequences for the passage behaviour of ^{13}C-labelled NDF. Therefore, the use of ^{13}C as an internal marker offers promising perspectives to acquire information on feedstuff and feed component specific fractional passage rates.

Key words: Rye grass, Silage, ^{13}C-labelling, Marker passage, Dairy cow

Introduction

International developments in feed evaluation for ruminants show a preference towards future use of systems of a more mechanistic and dynamic nature (Baldwin, 1995; AFRC, 1998). In the Netherlands similar ideas on this topic have already been worked out in a feasibility study (Tamminga *et al.*, 2000). In such systems, fractional passage rates form one of the most important factors as they determine the site and extent of degradation and the efficiency of microbial protein synthesis (Dijkstra and France, 1996). Hence, for future development of nutrient based feed evaluation systems, knowledge about feed specific retention times is essential information, which up-to this moment is largely lacking. To acquire information on feed specific fractional passage rates, external and internal markers

may be used. Internal markers are preferred, as they are more representative for the *in vivo* situation. The stable isotope of carbon (^{13}C) offers promising perspectives to study feed specific passage kinetics on the basis of the natural occurring differences in ^{13}C between cool season and warm season plant species (Südekum *et al.*, 1995) and by use of artificially labelled herbage (Svejcar *et al.*, 1993). Some studies report on labelling procedures (Svejcar *et al.*, 1990; Svejcar *et al.*, 1993; Thompson, 1996), but information on the allocation of the ^{13}C to different cell wall fractions of plants (NDF, ADF and ADL) and its possible implications for ruminant nutrition research are lacking.

The aim of the study was to develop a labelling procedure for rye grass (*Lolium perenne* L.) under field conditions that yields sufficient amounts of enriched material for use in passage studies in dairy cows. Furthermore, this paper will focus on the distribution of ^{13}C between different plant fractions and its implications when used as a marker in ruminant nutrition research.

Materials and Methods

Labelling Procedure

During the summer of 1997 and of 1998, four consecutive labelling experiments were conducted. In both seasons, uniform pastures were selected at 'De Ossekampen', the experimental farm of Wageningen University. The pastures were re-established with rye grass (*Lolium perenne* L) two years prior to the experiments. For each experiment two plots were selected, each with an area of 2.15 m^2 (155 × 139 cm). During the first year (Exp.1), the plots were labelled with $^{13}CO_2$ at 10 days during the regrowth period. Each labelling procedure started at 0900 h and lasted 1.5 to 2.0 h, provided that there was sufficient solar radiation for proper assimilation. At the onset of a labelling procedure, assimilation cages (155 × 139 × 30 cm) were placed over the plots. The sides of the cages were made of 2-cm clear Perspex and the topsides were covered with a sheet of transparent polyethylene. To ensure airtight sealing, the cages were placed in an iron U-profile that was anchored 5 to 10 cm into the soil. The U-profile contained a lactic acid solution (0.01 *M*, Merck 366) to prevent escape of CO_2. Inside each cage two electrical fans were placed in order to circulate the administered $^{13}CO_2$ through the cage. Prior to a labelling, for each cage 4.9 g (57.65 mmol) of [^{13}C]bicarbonate (99 atom%, Mass Trace) was weighed into a 250-mL glass bottle and dissolved in approximately 100 mL de-mineralized water. The bottle was sealed using a rubber stopper with an outlet connected to the interior of the chamber by means of a short plastic tube. The bottle was placed on a magnetic stirrer and subsequently every 10 min 10 mL of lactic acid (2 *M*) was added to the content of the bottle using a syringe. After adding a total of 40 mL, an additional 40-mL lactic acid was given to make sure all $^{13}CO_2$ was released. To ascertain that all $^{13}CO_2$ was expelled from the bottle and assimilated by the grass, 10 mL of bicarbonate (5 g/ 100 mL;

Table 1. Moment and length of the regrowth periods, the fertilizer (N) application, the surface area of labelled plots, number of labeling treatments, the amount of bicarbonate and ^{13}C administered during the experiments, the yield of grass silage (Exp.1, 3, 4) or grass (Exp.2) and the percentage of ^{13}C recovered in the harvested materials

	Exp.1	Exp.2	Exp.3	Exp.4
Regrowth period				
Start date	07-07-'97	12-05-'98	06-07-'98	06-07-'98
Harvest date	18-08-'97	24-06-'97	13-08-'98	24-09-'98
Treatments				
Fertilizer[a], kg N/ha	-	60	50	100
Plot area, m^2	4.31	2.15	4.31	4.31
No. of label days, d	10	4	6	8
[^{13}C]bicarbonate[b], g	98	32	110	140
^{13}C, (g)	14.990	4.895	16.826	21.414
Harvest				
Yield, g product	4155	7214	5305	7759
DM, g/kg	392.8	151.2	292.0	303.0
Recovery, % of ^{13}C	38.2	68.4	38.1	29.6

[a] Amount of N applied at the onset of the regrowth period.
[b] Bicarbonate [^{13}C, 99 atom%, $^{13}C_1$], MASSTRACE, Inc., 3-G Gill St. Woburn, MA 01801 USA.

Merck 6323) was added every 10 min during a 40-min period. Then, for the remainder of the day, the cages were removed from the plots. To restrict respiratory losses during the nights, the cages were placed back on the plots and removed the following morning. In the second year (Exp.2, 3 and 4), bad weather conditions prevented the exact repetition of the labelling procedure. Because of the limited days with adequate solar radiation the dosages of [^{13}C]bicarbonate were increased to 10.0 g per labelling as to decrease the number of days needed to obtain sufficient levels of enrichment. In order to release all $^{13}CO_2$ from the [^{13}C]bicarbonate an extra 40-mL of lactic acid was needed and, hence, increasing the total daily labelling procedure with 40 min. In Exp.2, 3 and 4, the plots received respectively, 4, 6 and 8 treatments with [^{13}C]bicarbonate during the regrowth period. Table 1 summarises growing conditions and treatments during the experiments.

Sampling and Chemical Analyses

In Exp.2 the process of labelling was monitored during the regrowth period. Pluck samples were taken from the labelled and unlabelled grass at days following a labelling treatment and at the day of harvest. For the sampling, vegetative shoots of grass were plucked at 2 cm above ground level. Samples were oven dried at 70°C and ground to pass a 1-mm screen (Wiley mill, T. Peppink & Zn., Machinefabriek, Amsterdam). Samples were subsequently ground in a bullet mill (Retsch MM 2000) for 5 min at 80 Hz and analysed for ^{13}C-enrichment using an isotope ratio mass spectrometer (Finnigan_MAT CN).

In Exp.1 and 2 plots and pasture were harvested at respectively two and one week after the final day of labelling. For Exp.3 and 4, plots and pastures were harvested the day following

the final labelling. The plots were cut by hand at 2 to 3 cm above ground level. Grass labelled in Exp.1, 3 and 4 was wilted and dried similar to the remainder of the pasture, placed in permeable nylon bags and ensiled with the bulk silage. After a 6-week ensiling period the ^{13}C-labelled material (GS1, GS3, GS4) was retrieved, cut to a length of about 3 cm using a paper cutter (Ideal 1035A), homogenised, placed in an airtight plastic bag and stored at – 20°C pending further analyses. The labelled grass obtained in Exp.2 (G2) was directly placed in an airtight plastic bag and stored at – 20°C pending further treatment and analyses. In addition to the labelled materials, samples were taken from the untreated silages (C1, C3, C4) and grass (C2) that were stored at – 20°C in airtight plastic bags.

Representative sub samples from the frozen materials were freeze-dried (FTS Dura-Dry programmable tray freeze drier) and ground to pass a 1-mm screen. Samples were analysed for DM, inorganic matter (Ash), NDF, ADF, ADL, neutral detergent residue (NDR), acid detergent residue (ADR) and acid detergent lignin residue (ADLR). Air-dried material, NDR, ADR and ADLR were subsequently ground in a bullet mill for 5 min at 80 Hz and analysed for total carbon content (TC) and ^{13}C-enrichment using an isotope ratio mass spectrometer. Results are presented as atom percentage ^{13}C (At%^{13}C) and delta ^{13}C (δ^{13}C). δ^{13}C expresses the ^{13}C:^{12}C ratio in a sample relative to the ^{13}C:^{12}C ratio of the international PDB standard (Pee Dee Belemnite, Eq.1).

$$\delta^{13}C = (\ ^{13}C:^{12}C \text{ of sample} - {}^{13}C:^{12}C \text{ of standard}) \times 1000 / {}^{13}C:^{12}C \text{ of standard} \qquad [1]$$

The At%^{13}C refers to the absolute number of ^{13}C atoms relative to the total number of C atoms (Eq.2).

$$At\%^{13}C = \text{no. of atoms } ^{13}C / \text{total no. of atoms C } (^{12}C, ^{13}C, ^{14}C) \times 100\% \qquad [2]$$

DM and Ash were determined in accordance with the respective ISO methods 6496 and 5984. ADF and ADL were analysed as described by Van Soest (1973). NDF was analysed according to a modified method of Van Soest (1991) as described by Goelema *et al.* (1998). The NDR, ADR and ADLR were analysed in accordance with the above mentioned methods, but excluding the incineration step to correct for the Ash content.

Calculations and Statistical Analyses

The At%^{13}C and δ^{13}C-values of the non-cell wall fractions, i.e. the neutral detergent solubles (NDS), were determined by quantifying the differences in the amount of ^{13}C between the DM and NDR relative to the differences in total amount of C between these fractions. The amount of ^{13}C in the fractions was corrected for the change in molecular weight inherent to the changes in the level of ^{13}C. TC in the NDS was corrected for the relative Ash content in the NDR fraction, based on NDF analyses. Cellulose and hemicellulose fractions were derived from the proximate Van Soest analyses by respectively, subtracting ADL from ADF

and ADF from NDF. In combination with the TC and At%^{13}C-values found in the NDR, ADR and ADLR, the level of enrichment in the cellulose and hemicellulose fraction could be established. Corrections for changes in molecular weight and differences in Ash content between fractions were included.

Levels of enrichment in the chemically determined fractions (DM, NDF, ADF, ADL) and assessed fractions (NDS, cellulose and hemicellulose) under naturally occurring conditions and after labelling were tested for differences using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC.). Within the statistical model the assessed fractions and their levels of enrichment are treated as independent factors. The model includes the main effects Treatment (labelled vs. unlabelled; df1), Fractions (df6) and Exp. (df3) and the two-way interaction terms (Eq.3).

$$Y_{ijk} = \mu + T_i + F_j + E_k + (T \times F)_{ij} + (T \times E)_{ik} + (F \times E)_{jk} + e_{ijk} \quad [3]$$

where Y_{ij} represents the dependent variable, μ represents the mean, T_i corrects for the treatment effect (i = 1, 2) F_j corrects for the fraction effect (j = 1 to 7), and E_k corrects for the experiment effect (k = 1 to 4), $(T \times F)_{ij}$, $(T \times E)_{ik}$, and $(F \times E)_{jk}$ refer to the interactions and e_{ijk} denotes the error term.

Results and Discussion

Qualitative Aspects of Labelling

During Exp.2 qualitative pluck samples were taken from the plots and unlabelled pasture at days following a labelling treatment. The levels of enrichment of pluck samples following these labelling days (symbol Δ, d1 to d4) or at the moment of harvest (symbol Δ, d5) are shown in Figure 1. δ^{13}C-values of respective samples (68.9, 190.0, 340.1, 629.0 and 572.7‰ vs. PDB) show a strong increase after each labelling day and a slight decrease between the final labelling and the moment of harvest. Samples obtained from the unlabelled pasture represent the background level of enrichment (symbol ▼, d1` to d5`; Figure 1), with an average δ^{13}C-value of –30.55‰ vs. PDB (SD = 0.75‰). The relative increase in enrichment between the successive four labelling days equals 99.1 (d1 – d1`), 120.9 (d2 – d1), 150.0 (d3 – d2), 290.3 (d4 – d3) ‰ vs. PDB. At the first labelling day 5 g of [^{13}C]bicarbonate was given. For the following labelling days (2, 3 and 4) the amounts of [^{13}C]bicarbonate were increased to respectively, 7, 10 and 10 g, adding up to a total of 32 g. The increase of label administration explains the higher levels of enrichment after the second and third labelling, however, the increase after the fourth labelling day suggests a more efficient fixation of ^{13}C by the plants at d4 compared to d3. As weather conditions were very unstable at the time of the regrowth period, especially at the moment of labelling days 1, 2, and 3, this could well have an effect on the efficiency of the labelling process.

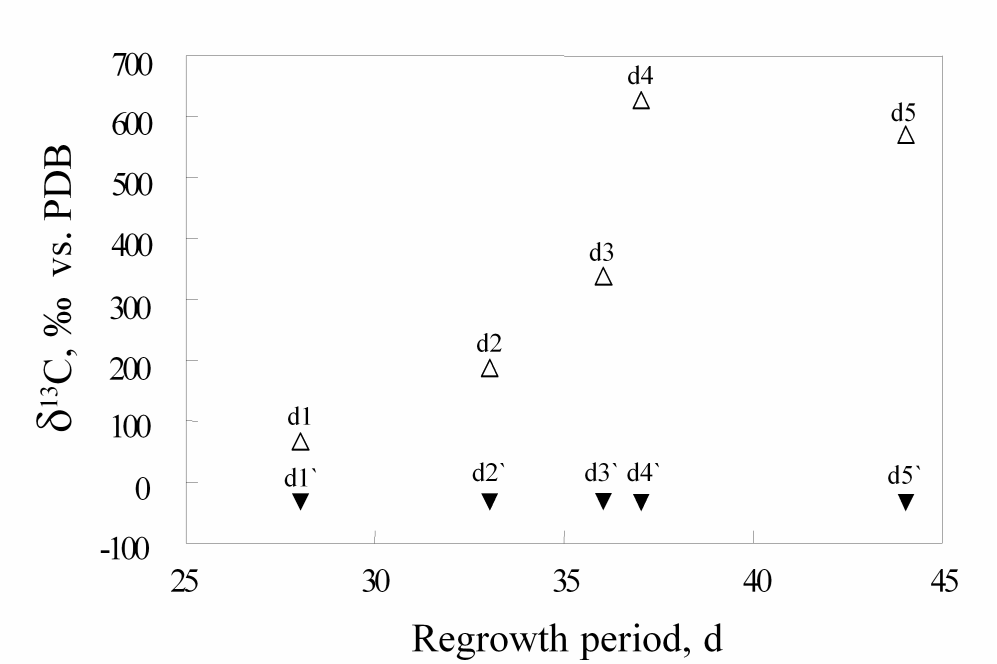

Figure 1. $\delta^{13}C$ values of labelled (Δ) grass after four successive labelling procedures (d1 to d4) and at the moment of harvest (d5). The naturally occurring level of enrichment in unlabelled grass (▼) is represented by d1` to d5`

Quantitative Aspects of Labelling

Efficiency of ^{13}C-utilization. Table 2 summarises cell wall composition and the enrichment in the DM of the labelled and unlabelled materials. The actual DM harvested from the labelled plots GS1, G2, GS3 and GS4 were respectively 1632.1, 1090.8, 1549.1 and 2351.0 g, the equivalent of 3788, 5063, 3595 and 5456 kg DM/ha (Table 1). The amount of extra ^{13}C assimilated by the plants was derived from the difference in enrichment between the labelled and unlabelled materials. For GS1 the total C fixation was 739.0 g. After correcting for the level of enrichment under natural conditions (At%^{13}C = 1.0786) and the increase in atomic weight (gram atoms; gat) due to the elevated levels of ^{13}C after labelling (unlabelled = 12.0108 gat and labelled = 12.0180 gat), the total amount of ^{13}C fixed by GS1 was 14.353 g. Corrected for the amount of ^{13}C naturally present in grass (8.627 g ^{13}C), this results in an extra accumulation of ^{13}C in the above ground plant parts of 5.726 g. During the total period of labelling 98 g [^{13}C]bicarbonate was used that corresponds with 14.990 g of ^{13}C. This results in a recovery of the ^{13}C-label in the above ground plant parts of 38.2% (Table 1), which is slightly higher than findings reported by Svejcar *et al.*, (1990). In an experiment with cheatgrass (*Bromus tectorum* L.) they quantified the allocation of excess ^{13}C to the roots and respiratory losses to be as high as 66%. The recoveries of ^{13}C in the other experiments were estimated in a similar way resulting in recovery percentages of 68.4, 38.1 and 29.6% for respectively G2, GS3 and GS4. The high level of ^{13}C-fixation for G2 can be partly attributed to low levels of translocation of C from the shoots to the roots. Cornish (1987) reported that plants emphasize the development of the above ground plant parts at the expense of the roots when water supply by the soil is adequate. As a direct effect, the absolute C-partitioning to the

Table 2. Cell wall characteristics (NDF, ADF, ADL) and the total C content (TC) and isotope ratios (At%^{13}C, δ^{13}C) in the DM and cell wall fractions of labelled and unlabelled materials

	Labelled materials				Unlabelled materials			
Item	GS1	G2	GS3	GS4	C1	C2	C3	C4
Isotope ratio analyses in the DM								
OM, g/kg DM	905.0	899.2	881.4	897.4	904.2	905.3	888.6	878.4
TC, % in DM	45.28	43.52	43.10	43.32	45.34	44.09	45.28	44.24
At%^{13}C, %	1.7955	1.7296	1.9681	1.6541	1.0786	1.0770	1.0789	1.0783
δ^{13}C, ‰ vs. PDB	627.1	566.3	786.6	496.7	-29.7	-31.1	-29.4	-30.0
Isotope ratio analyses in the NDF[a]								
NDF, g/kg DM	452.6	488.2	432.7	523.9	467.7	520.0	409.4	510.5
TC[b], %	45.99	45.75	45.76	46.19	46.75	46.16	45.82	46.25
At%^{13}C, %	1.8913	1.7102	1.8833	1.5668	1.0791	1.0776	1.0793	1.0792
δ^{13}C, ‰ vs. PDB	715.5	548.4	708.1	416.4	-29.2	-30.6	-29.0	-29.1
Isotope ratio analyses in the ADF[a]								
ADF, g/kg DM	269.2	289.7	280.4	322.3	282.7	304.5	262.5	305.1
TC[b], %	44.61	43.91	44.00	44.62	45.51	43.77	44.30	45.29
At%^{13}C, %	1.8843	1.6770	1.8571	1.5400	1.0796	1.0776	1.0810	1.0802
δ^{13}C, ‰ vs. PDB	709.1	517.8	683.9	391.9	-28.7	-30.6	-27.5	-28.2
Isotope ratio analyses in the ADL[a]								
ADL, g/kg DM	24.1	25.4	22.7	35.4	24.4	23.8	21.3	29.6
TC[b], %	36.94	51.46	46.02	54.40	42.85	44.09	43.35	48.38
At%^{13}C, %	1.6525	1.5654	1.5810	1.3487	1.0787	1.0764	1.0757	1.0764
δ^{13}C, ‰ vs. PDB	495.3	415.2	429.5	216.6	-29.6	-31.7	-32.3	-31.7

[a] TC and isotope ratios determined in the residues NDR = Neutral Detergent Residue; ADR = Acid Detergent Residue; ADLR = Acid Detergent Lignin Residue.
[b] TC expressed as % of the fractions (NDR, ADR, ADLR) after drying at 103°C, and followed by a correction for ash content.

above ground parts will be higher. Brougham (1959), Alberda and Sibma (1968), Robson (1973) and Simpson and Culvenor (1987) also reported increased DM yields under conditions of moderate ambient temperatures and high radiation inputs. They related this to a decrease in C-loss by respiration. The period after the fourth labelling day in Exp.2 (Figure 1; d4 to d5) was characterised by high levels of rainfall, moderate temperatures and long day lengths. The combination of these conditions likely forms an important factor for the high level of C-recovery in G2. Furthermore, labelled material was directly frozen after cutting and not ensiled like GS1, GS3 and GS4. Storage losses that occur during the ensiling period can be as high as 15% of the DM (Wilkinson, 1981). These losses can be ascribed to respiration, anaerobic fermentation, effluent and surface waste. The respiration and anaerobic fermentation processes will use part of the readily soluble carbohydrates within the cell content and hence, partly contribute to the decrease in ^{13}C recovery of the ensiled materials (GS3 and GS4). In addition, the relatively lower ^{13}C-recovery percentages in these silages can be partly explained by the means of label application. Unstable weather conditions enforced us to label at consecutive days and to increase the amount of label. The short intervals between successive labelling days may have resulted in relatively higher ^{13}C-losses due to overnight respiration. Although cages were placed on the plots overnight, in these cases they had to be removed earlier in the morning in preparation of the next labelling treatment.

Labelling of cell wall constituents. The cell wall composition (NDF, ADF, ADL) and the distribution of ^{13}C between these fractions are shown in Table 2. Comparing the level of enrichment in the NDF with that in the DM shows that the DM enrichment is higher in G2, GS3, GS4 but lower in GS1. Similarly, only for GS1 was the enrichment of DM lower than that of ADR. The lower level of enrichment of DM in GS1 can be explained by the extended regrowth period of two weeks between the final labelling and the moment of harvest. This agrees with the findings of Svejcar *et al.* (1993) who observed lower enrichment values in the cell content compared to whole plant material when harvesting alfalfa 1 month after labelling. A delay in the moment of harvest relative to the moment of labelling causes a dilution of ^{13}C in the total C-pool of the non cell wall fraction (NDS) and hence, increases the relative level of enrichment of the cell wall fraction.

Table 3 presents the least square means of the analytically determined (OM, NDF, ADF, ADL) and arithmetically determined (NDS, hemicellulose, cellulose) plant fractions, their total C-content (TC) and the level of ^{13}C-enrichment for labelled and unlabelled materials. No differences were observed in the quantities and TC-values of fractions between the labelled and unlabelled materials ($P \geq 0.737$), which suggests that the labelling treatments did not affect plant composition. The labelling treatments did increase the level of enrichment for all fractions ($P < 0.001$). With regard to the $\delta^{13}C$-values of grasses grown under natural conditions no statistical differences were observed between fractions ($P = 1.000$). The labelled grasses showed considerable differences in the level of enrichment between fractions, which suggest that isotope fractionation against ^{13}C occurs with regard to the lignin fraction

Table 3. The amount, total C content (TC) and isotopic ratios ($\delta^{13}C$) in the DM, NDF, ADF, ADL, NDS, hemicellulose and cellulose fractions of unlabelled (U) and labelled (L) materials[a]

Item	Amount		TC[b]		$\delta^{13}C$	
	U	L	U	L	U	L
OM[c]	894.1^{u}	895.8^{u}	44.74	43.81^{uv}	-30.05	619.18^{u,***}
NDF	476.9^{v}	474.4^{v}	46.25	45.92^{uv}	-29.48	597.10^{u,***}
ADF	288.7^{w}	290.4^{w}	44.72	44.29^{uv}	-28.75	575.68^{u,***}
ADL	24.8^{x}	26.9^{x}	44.67	47.21^{uv}	-31.33	389.15^{v,***}
NDS	523.1^{y}	525.7^{y}	44.03	42.60^{u}	-30.60	647.28^{u,***}
Hemicellulose	188.2^{z}	184.0^{z}	48.60	48.55^{v}	-30.65	628.40^{u,***}
Cellulose	264.0^{w}	263.5^{w}	44.71	43.95^{uv}	-28.53	595.60^{u,***}
SEM	6.1		0.89		23.59	
Model evaluation[d]						
rMSE	12.3		1.77		47.18	
Treat (I)	0.973		0.677		<.001	
Fraction (II)	<.001		<.001		0.001	
Exp (III)	0.001		0.581		<.001	
(I×II)	0.997		0.424		0.001	
(I×III)	0.364		0.566		<.001	
(II×II)	<.001		0.018		0.495	

[a] Values presented as least square means; Amounts expressed in g/kg DM; TC = Total C content (% within the fraction); $\delta^{13}C$ = level of isotopic enrichment in ‰ vs. PDB.

[b] TC corrected for differences in Ash content in fractions.

[c] TC and $\delta^{13}C$-values reflect those obtained in the DM.

[d] Statistical model includes the main effects Treat (1df), Fraction (df6), Exp. (df3), and their two-way interaction terms; SEM = pooled standard error of mean; rMSE = root mean square error;

Differences between treatments (U vs. L) indicated by; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

^{u,v,w,x,y,z} Within columns mean values differ ($P < 0.05$).

as determined by the proximate Van Soest analyses. This corresponds with the observations of Benner *et al.* (1987), who reported lower levels for δ^{13} (2 to 6‰-units) in the ADL fraction compared to whole plant tissue in both C_3 and C_4 plant species grown under natural conditions, whilst the cellulose and hemicellulose fractions had a 1 to 2‰ higher $\delta^{13}C$-value. Between the fractions in our unlabelled materials the differences in enrichment were similar but less pronounced. With regard to the $\delta^{13}C$-values observed in labelled materials, ADL had a 230‰-unit lower $\delta^{13}C$-value compared to the DM fraction ($P = 0.0001$), hemicellulose had a 9‰-unit higher $\delta^{13}C$-value ($P = 1.000$), whilst cellulose had a 24‰-unit lower enrichment ($P = 1.000$). These results show that the effect of discrimination against ^{13}C in the lignin fraction is more pronounced when the plant is growing under elevated levels of ^{13}C. According to Benner *et al.* (1987) the fractionation against ^{13}C occurs during the formation of the biosynthetic precursors of lignin, phenylalanine and tyrosine. Tyrosine is an important

precursor of lignin in graminaceous species and is known to be relatively more depleted in ^{13}C compared to phenylalanine. This likely explains the large difference in $\delta^{13}C$ in ADL compared to the other fractions as observed in the current work. The low level of $\delta^{13}C$ in ADL relative to the DM was compensated by an increase in $\delta^{13}C$ (28‰-units) of the non cell wall fraction, whilst within the cell wall fraction cellulose even gave a lower value for $\delta^{13}C$ compared to the DM. This was not anticipated from literature, and our data also suggests that the process of fractionation against ^{13}C during cell wall formation is likely to be more variable than what has been shown by Benner *et al.* (1987).

When $\delta^{13}C$ -values in the NDF, ADF and ADL are expressed relative to those of the DM, the ADL has a distinct lower enrichment for GS1, G2, GS3 and GS4 of respectively 21, 27, 45 and 56%. GS1 had a 13 to 14% higher enrichment in NDF and ADF whilst G2, GS3 and GS4 had lower enrichments compared to the DM, ranging from 3 to 21% lower values. These differences can be partly explained by the differences in labelling treatments between experiments. GS1 received 10 treatments with 4.9 g of [^{13}C]bicarbonate between day 17 and day 29 of regrowth. In contrast GS4 received 8 treatments (of which 6 with 10.0 g [^{13}C]bicarbonate) between day 45 and day 80 of regrowth. During the labelling treatments GS4 was in a much more physiological mature stage, which is also reflected in the higher NDF, ADF and ADL content compared to the other grasses. This may have been an important factor contributing to the low levels of enrichment and heterogenous distribution of label between cell wall fractions. Also the moment of the season in combination with weather conditions seem to be contributing factors to the success of labelling treatment. Although differences exist between the distribution of ^{13}C in the DM and NDR, and within the NDR between the hemicellulose and cellulose, no statistical indication was found for discrimination against ^{13}C, i.e., the partitioning of the artificially administered ^{13}C to these fractions can be assumed homogenous. This agrees with the observations of Smith *et al.* (1986) who found a uniform distribution of ^{14}C in DM compared to NDF of lucerne after four labelling treatments, with respective values for specific activity of 4,000 dpm/ mg DM and 4,332 (± 319) dpm/ mg NDF.

Labelling grass for passage studies. Our main aim was to obtain sufficient amounts of labelled materials for use in passage studies in dairy cows. A constraint arising here can be the financial feasibility because of the costs involved when using stable isotopes. To assess the desired level of enrichment of the plots, first the minimum amount of marker material required to label a rumen pool was estimated using the multicompartmental model (Eq.4) as proposed by Dhanoa *et al.* (1985).

$$Y = A e^{-(K_1 \times t)} \exp\left[-(N-2)\, e^{-(K_2 - K_1) \times t}\right] \qquad [4]$$

where Y refers to the faecal marker concentration at time = t, N denotes the number of compartments and K_1 and K_2 represent the rate constants for the two compartments in the digestive tract with the longest retention times. A forms a scalable parameter dependent on K_1,

K_2 and N. In further estimations we assumed A to represent the initial level of enrichment above natural enrichment (atom percentage excess, APE) in the rumen DM compartment.

The accuracy of an Isotope Ratio Mass Spectrometer (IRMS) allows to detect differences of 1 δ^{13}C (0.0011 APE) between samples. The total clearance time ($t = t_{(tc)}$), the moment when a marker is completely removed from the digestive tract, was set at $t_{(tc)}$ = 120 h after pulse dose administration. This was based on a pilot trial where Cr-mordanted fiber was used as a particle phase marker (our unpublished observations). Therefore, at time $t_{(tc)}$ the level of enrichment in the faecal DM should be back to the level of natural enrichment, e.g., 1.0786 At%^{13}C. This level of natural enrichment in the digestive tract occurs when animal diets are fully composed of C_3-plant species (At%^{13}C in DM of C1; Table 2). Faecal DM at time $t_{(tc-t)}$ = 96 h was assumed to have a marker concentration of 0.0011 APE. Parameter setting of K_1, K_2 and N was done using data from a study reported by Dhanoa *et al.* (1985) where cows received pulse doses of Cr-mordanted precision-chopped silage in the rumen. With set values for K_1 = 0.0258/h, K_2 = 0.1785/h and N = 10 an initial value for A was obtained of 0.0131 APE, resulting in a level of enrichment of the DM rumen pool of 1.0917 At%^{13}C. Assuming a DM pool of 12 kg and a TC of 45.0% in this pool results under natural enrichment conditions in 449.60 gat of total C in the rumen (5400 g C/ 12.0108 mol). We further set the size of the pulse dose at 2% of the rumen pool DM weight (240 g DM of enriched material; TC = 45%). To enrich the rumen pool to 1.0917 At%^{13}C, an extra amount of 0.1570 gat ^{13}C and 8.8299 gat ^{12}C needs to be added to the rumen, resulting in a relative enrichment of the pulse dosed grass of 1.7465 At%^{13}C. An experimental design with 6 rumen pools to be enriched requires 1440 g of enriched DM (3341 kg DM/ha). The two assimilation cages used in our experiments cover an area of 2.15 m^2 each. Assuming a yield per ha of 3341 kg DM at the moment of harvest results in a total C-fixation on both plots of 648.0 g C (TC = 45.0%). Under natural conditions 0.5819 gat ^{13}C (7.57 g ^{13}C) will be incorporated by the grass. To obtain a target enrichment of 1.7465 At%^{13}C an extra amount of 0.3598 gat ^{13}C (4.68 g ^{13}C) needs to be incorporated by substituting the ^{12}C-fraction. In combination with a recovery percentage of 34% (Svejcar *et al.*, 1990) a total of 13.76 g ^{13}C would be required for the labelling procedure, which corresponds with 89.94 g [^{13}C]bicarbonate. Table 4 summarises the results of a sensitivity analyses for A, the required amounts of [^{13}C]bicarbonate and the At%^{13}C in relation to a change in time interval between $t_{(tc)}$ (120 h) and $t_{(tc-t)}$ (96 or 108 h) where the minimum difference is fixed at 1 δ^{13}C. The numbers of curves are based on actual experiments (our unpublished observations). The overall recovery of ^{13}C-label in the above ground plant parts is arbitrarily set at 34%. The average DM yields per ha are set in accordance with the guidelines from the Dutch Information and Knowledge Center for Animal Husbandry (Anonymous, 1993). These guidelines are based on regression equations that include the date of previous harvest, the amount of applied fertilizer and the length of the regrowth period. When comparing these projected yields with the actual DM yields of the plots (Table 1) it appears that the yields for G2 and GS4 were much higher than anticipated. Due to poor climatic conditions at the moment of Exp.2 and Exp.4 the number of days with

Table 4. Summary of the initial model assumptions regarding field and animal experiments, the estimations for the desired level of enrichment for different time intervals and accompanying required amounts of [^{13}C]bicarbonate[a]

Item	GS1	G2	GS3	GS4
	Initial settings			
Field experiments				
Plot area, m^2	4.31	2.15	4.31	4.31
Yield per ha, kg DM	3341	2000	3000	4000
Yield per plot, g	1440.0	430.0	1293.0	1724.0
TC, %		45.0		
At%^{13}C, %[b]		1.0786		
^{13}C recovery, %		34.0		
Animal experiments				
No. of pulse dose treatments	6	2	6	6
A at $t_{(tc-t)}$ = 96 h, APE[c]	0.01309	0.01309	0.01309	0.01309
A at $t_{(tc-t)}$ = 108 h, APE[c]	0.01784	0.01784	0.01784	0.01784
	Estimations for grass enrichment			
One δ^{13}C-value difference at $t_{(tc-t)}$ = 96 h				
[^{13}C]bicarbonate, g	89.9	29.9	89.8	90.3
Target At%^{13}C, %	1.7465	1.8227	1.8210	1.6386
One δ^{13}C-value difference at $t_{(tc-t)}$ = 108 h				
[^{13}C]bicarbonate, g	122.6	40.8	122.3	123.1
Target At%^{13}C, %	1.9891	2.0929	2.0906	1.8419

[a] Multicompartmental model is used as described by Dhanoa *et al.* (1985), initial values for K_1, K_2, and N within the range of values reported by Dhanoa *et al.* (1985).
[b] Assumed value for naturally occurring enrichment of grass.
[c] *A* represents the initial concentration of the rumen pool with total marker recovery set at time $t_{(tc)}$ = 120 h after pulse dose and $t_{(tc-t)}$ = 96 or 108 h, where $t_{(tc-t)}$ denotes the one before last collected faecal sample.

sufficient solar radiation required for labelling was sparse and dates of harvest had to be delayed. With respect to the initial settings and the higher actual DM yields it is likely that the ^{13}C administered gets more diluted, hence, results in a lower enrichment value. However, because of the high ^{13}C recovery in G2 an acceptable level of enrichment was achieved. For GS4 the labelling procedure resulted also in an acceptable level of enrichment but here the At%^{13}C was lower than aimed for with regard to the actual amount of [^{13}C]bicarbonate used.

After obtaining the actual amounts of labelled material, the height of the ruminal pulse doses can be optimized by creating and assessing fictional ^{13}C-excretion patterns using Eq.4. A difficulty is to sufficiently enrich the pool size to be able to distinguish an excretion pattern, as the amount of available marker is limiting. In a study with steers receiving a single meal pulse dose of maize silage (C_4-species) in a whole-plant barley silage diet (C_3-species), Südekum *et al.* (1995) observed faecal excretion patterns with differences between peak

enrichment and the level of enrichment at the end of the curve of 3 to 4 δ^{13}C-units. Assuming that this difference between peak and background level of enrichment would suffice, model approximations were evaluated for maximum possible levels of pulse dosed materials and for actual pulse dosed materials. The maximum levels were calculated from the amount of harvested enriched material (Table 1, 2) relative to the number of pulse dose treatments and the actual amounts on animal experiments (our unpublished observations). Values for K_1 and K_2 were fixed at respectively 0.0258/h and 0.1758/h and N was set at 10, 30 or 60. An increase in N results in lower estimates for the peak concentration and increases the time interval when peak concentration is reached (Table 5; Figure 2). In case of GS1 the actual amount used appears to be on the low side. Therefore, in the follow up trial where this silage was tested ruminal pulse doses were increased from 175 g to 234 g DM.

Considerations for animal nutrition research

In digesta passage studies stable isotopes like ^{13}C (Svejcar *et al.*, 1993; Südekum *et al.*, 1995) and ^{15}N (Huhtanen and Hristov, 2001) form promising tools to acquire information of feed component specific fractional passage rates. It may be assumed that these isotopes behave as ideal flow markers as they form intrinsic constituents of the feed fractions. However, a potential problem to accurately assess the passage kinetics is the isotopes are incorporated in both the digestible an indigestible fractions. An additional complication is when a component (e.g. NDF) consists of more fractions (hemicellulose, cellulose, lignin) with different digestibilities and levels of enrichment. Huhtanen and Hristov (2001) tried to circumvent this by labelling the low degradable part of the fiber bound N fraction (ADF-N) with ^{15}N, and compared its passage with that of ^{15}N-labelled NDF (NDF-^{15}N). They observed a shorter rumen retention time for NDF-^{15}N compared to ADF-^{15}N, which they attributed to the partial digestion of the fiber-bound N. However, it can be hypothesised that the processes of degradation do not (or only moderately) discriminate against one of the isotopes (^{15}N or ^{14}N), but certainly will not have an absolute preference for ^{15}N. Therefore, it may be assumed that both isotopes will disappear (degrade) proportionally, and hence, isotope ratio will not be directly affected as such. It is more likely that the difference between NDF-^{15}N and ADF-^{15}N passage is related to a 'dilution' of the cell wall associated ^{15}N via microbial contamination. Although contamination is minor, relative to the absolute but small amount of fiber bound N its effect will be considerable, and therefore, makes NDF-^{15}N unsuitable as a marker. By using ^{13}C to label the NDF fraction the effect of dilution through microbial contamination will probably lie outside the detectable range, and hence, may be assumed to have no effect on the fractional passage.

The discrimination against artificially administered ^{13}C related to the biosynthesis of the lignin precursors remains a point of consideration. Compared to the ADL/ NDF-ratio in the labelled materials (26.9/ 474.4 = 0.06; Table 3) in the distal parts of the digestive tract this ratio will increase. In case of an overall NDF digestibility of 0.70 and no lignin disappearance the ADL/ NDF-ratio would increase with 0.13 to a ratio of 0.19, which will influence the

Table 5. The potential (Maximized) amounts and the actual amounts of labelled materials (GS1, G2, GS3, GS4) used for pulse dosing into the rumen, the initial enrichment of the rumen pool, the actual peak enrichment and the peak enrichment after correction for natural enrichment ($\Delta(C_P-C_N)$)

	Maximized amount of pulse dose[a]				Actual amount of pulse dose[b]			
Item	GS1	G2	GS3	GS4	GS1	G2	GS3	GS4
				Rumen compartment[c]				
Pulse dose, g DM	272	545	258	392	175	227	180	260
At%^{13}C, %	1.0945	1.1053	1.0976	1.0965	1.0889	1.0891	1.0920	1.0905
APE, %	0.0159	0.0284	0.0187	0.0182	0.0103	0.0121	0.0131	0.0122
				Faecal excretion				
Enrichment at peak[d, f]								
N = 10, ‰	-23.35	-19.82	-21.94	-22.69	-25.58	-26.31	-24.17	-25.09
N = 30, ‰	-24.56	-21.99	-23.38	-24.08	-26.37	-27.24	-25.18	-26.02
N = 60, ‰	-25.16	-23.05	-24.08	-24.77	-26.75	-27.69	-25.67	-26.48
$\Delta(C_P-C_N)$[e, f]								
N = 10, ‰	5.72	10.19	6.73	6.55	3.71	4.36	4.73	4.39
N = 30, ‰	4.50	8.03	5.30	5.16	2.92	3.43	3.72	3.46
N = 60, ‰	3.90	6.96	4.60	4.47	2.53	2.98	3.23	3.00

[a] Amounts of pulse doses based on the total harvested amount of labelled materials (Table 1) relative to the no. of intended pulse dose treatments (Table 5).

[b] Amount based on actual experiments (Pellikaan *et al.*, 2004; Our unpublished observations).

[c] Values of At%^{13}C equals the enrichment in the rumen directly following from a pulse dose. APE (atom percentage excess) resembles *A* that represents the initial excess enrichment in the rumen pool.

[d] Peak level of enrichment in the faeces (δ^{13}C in ‰ vs. PDB).

[e] Units of δ^{13}C in ‰ vs. PDB.

[f] Values of *N* set at 10, 30 and 60 are within the range as reported by Dhanoa *et al.* (1985).

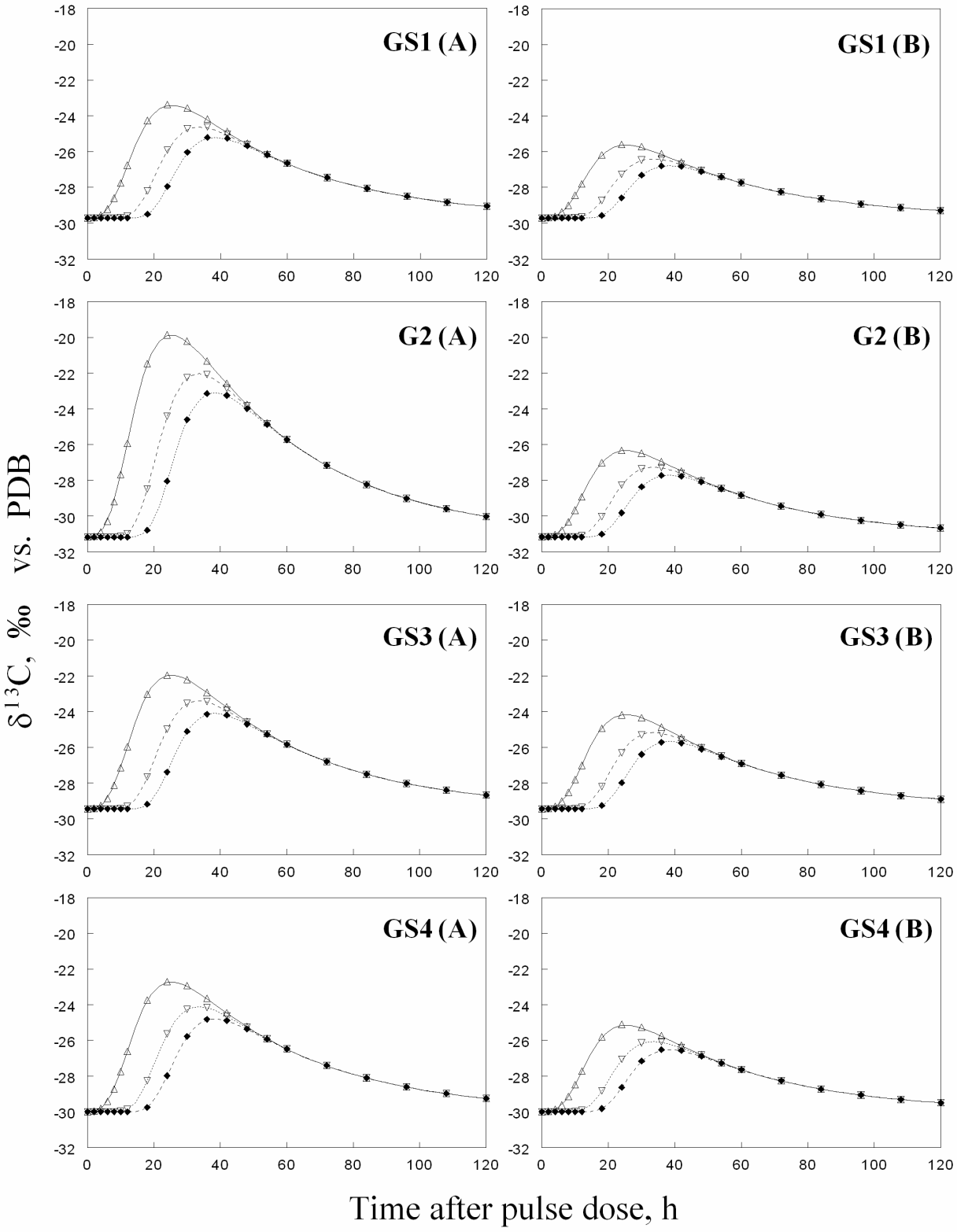

Figure 2. Faecal excretion pattern for the labelled silages (GS1, GS3, GS4) and grass (G2) at maximized level of pulse dosed material (**A**) or at the actual level of pulse dosed materials (**B**), for *N*= 10 (—Δ—), *N* = 30 (– –∇– –) and *N* = 60 (— —♦— —)

level of $\delta^{13}C$ because ADL has a 35% lower level of $\delta^{13}C$ (208‰-units) compared to NDF (Table 3). The effective change in enrichment caused by a shift in ADL/ NDF-ratio would result in an error of about 5% (35% × 0.13) relative to the NDF enrichment. This effect can be quantified using multiple canulated animals and compare excretion patterns of ^{13}C-labelled NDF, ADF and ADL at different places in the digestive tract. However, experimental and analytical variability will most likely prevent detection of such an error.

Implications

The labelling procedure of rye grass under field conditions described here is easy to repeat and yields adequate amounts of enriched materials to be used for passage studies in dairy cows. Four labelling treatments seem to be sufficient for proper labelling of different plant fractions, however, based on the variability in ^{13}C-allocation to fractions between experiments more treatments are advisable. Labelling grass at a late mature stage resulted in relative low levels of enrichment and more heterogeneous distribution of marker between cell wall fractions. The changes in ADL/ NDF-ratio due to degradation processes have probably negligible consequences for the passage behaviour. Therefore, the use of ^{13}C as an internal marker offers promising perspectives to acquire information on feedstuff and feed component specific fractional passage rates.

Acknowledgements

The authors kindly acknowledge the assistance of Mr. B. Tas, Mr. L. Bijl, Mr. A. van den Dool, Mr. R. Wormgoor, Mr. W. Nusselder, Mr. J. van Kleef, Dr. R. van Loo and Dr. J. Vos.

Literature Cited

AFRC, 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. AFRC Technical Committee on Responses to Nutrients. Report no. 11. Nutr. Abstr. Rev. (Ser. B) 68: 937-989.

Alberda, T. and L. Sibma, 1968. Dry matter production and light interception of crop surfaces. 3. Actual herbage production in different years as compared with potential values. J. Brit. Grassl. Soc. 23: 206-215.

Anonymous, 1993. Handboek rundveehouderij. Publicatie nr. 35. Informatie en Kennis Centrum Veehouderij, Afdeling Rundvee-, Schapen- en Paardenhouderij, Lelystad, The Netherlands.

Baldwin, R.L. 1995. Modeling ruminant digestion and metabolism. Chapman and Hall, New York.

Benner, R., M. L. Fogel, E. Kent Sprague, and R. E. Hodson. 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature. 329:708-710.

Brougham, R.W., 1959. The effects of frequency and intensity of grazing on the productivity of a pasture of short-rotation ryegrass and red and white clover. N.Z. J. Agric. Res. 2: 1232-1248.

Cornish, P.S., 1987. Root growth and function in temperate pastures In: J.L. Wheeler, C.J. Pearson and G.E. Robards (ed.) Temperate pastures, their production, use and management. p 609 Australian Wool Corporation/ CSIRO, Australia.)

Dhanoa, M.S., R.C. Siddons, J. France and D.L. Gale, 1985. A multicompartmental model to describe marker excretion patterns in ruminant faeces. Br. J. Nutr. 53: 663-671.

Dijkstra, J and J. France, 1996. A comparative evaluation of models of whole rumen function. Ann. Zootechnie., 45: 175-192.

Goelema , J.O., M.A.M. Spreeuwenberg, G. Hof, A.F.B. van der Poel and S. Tamminga,. 1998. Effect of pressure toasting on the rumen degradability and intestinal degradability of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Anim. Feed Sci. Technol. 76: 35-50.

Pellikaan, W.F., J. Dijkstra, S. Tamminga, H. Boer, S.C.W. Lammers-Wienhoven, W.J.H. van Gestel, and G. Hof. 2004. Passage of ^{13}C-labelled grass silage through the gastro-intestinal tract of dairy cows at two levels of feed intake. Submitted to JAS as companion paper.

Robson, M.J., 1973. The growth and development of simulated swards of perennial ryegrass. I. Leaf growth and dry weight change as related to the ceiling yield of a seedling sward. Ann. Bot. 37: 487-500.

Simpson, R.J. and R.A. Culvenor, 1987. Photosynthesis, carbon partitioning and herbage yield. In: J.L. Wheeler, C.J. Pearson and G.E. Robards (ed.) Temperate pastures, their production, use and management. p 609 Australian Wool Corporation/ CSIRO, Australia.

Smith, L.W. and R.A. Erdman, 1986. Production of alfalfa containing ^{14}carbon. J. Anim. Sci. 63 (Suppl. 1): 316 (Abstr.).

Südekum, K.H., W. Ziggers, N. Roos, H. Sick, S. Tamminga and M. Stangassinger, 1995. Estimating the passage of digesta in steers and wethers using the ratio of ^{13}C to ^{12}C and titanium(IV)-oxide. Isotopes Environ. Health Stud. 31: 219-227.

Svejcar, T.J., T.W. Boutton and J.D. Trent, 1990. Assessment of carbon allocation with stable carbon isotope labeling. Agron. J. 82: 18-21.

Svejcar T.J, M.B. Judkins and T.W. Boutton, 1993. Technical note: labeling of forages with ^{13}C for nutrition and metabolism studies. J. Anim. Sci. 71: 1320-1325.

Tamminga, S., W. Gerrits, A. Bannink, J. Dijkstra, G. Meijer en A. van Vuuren, 2000. Een opzet voor een nieuw, op nutriënten gebaseerd voederwaarderingssysteem voor herkauwers. In: 25^{e} Studiedag nederlandstalige voedingsonderzoekers, Wageningen, The Netherlands: 20-21 (Abstr.).

Thompson, R.B., 1996. Pulse-labelling a cover crop with ^{13}C to follow its decomposition in soil under field conditions. Plant Soil 180: 49-55.

Van Soest, P.J., 1973. Collaborative study of acid-detergent fiber and lignin. J. Assoc. Offic. Anal. Chem. 56: 781-784.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Wilkinson, J.M., 1981. Losses in the conservation and utilization of grass and forage crops. Ann. Appl. Biol. 98: 365-375.

Chapter 3

Passage of ^{13}C-Labelled Grass Silage Through the Gastro-Intestinal Tract of Dairy Cows at Two Levels of Feed Intake

W.F. Pellikaan[*], J. Dijkstra[*], S. Tamminga[*], H. Boer[*], S.C.W. Lammers-Wienhoven[†], W.J.H. van Gestel[*], and G. Hof[*,1]

[*]Animal Nutrition Group and [†]Human and Animal Physiology Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands

Submitted to Journal of Animal Science

[1] in memory of G. Hof

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W.F. Pellikaan, J. Dijkstra, S. Tamminga, H. Boer, S.C.W. Lammers-Wienhoven, W.J.H. van Gestel, and G. Hof

Abstract

Feed specific passage rates of grass silage were determined in different compartments of the gastro-intestinal tract of dairy cows assigned to a high (Exp.1) and low (Exp.2) level of feed intake using ^{13}C as an internal marker. In two experiments, three rumen and ileum cannulated HF cows received consecutive pulse doses of ^{13}C-labelled grass silage, Cr-NDF and Co-EDTA into the ileum, the abomasum and the rumen. In both experiments animal received diets with a fixed grass silage to concentrate ratio (60:40). Following pulse doses into the ileum, abomasum and rumen, faecal marker excretion patterns were determined. In addition the ileal marker excretion was followed after ruminal pulse doses. With regard to the passage characteristics of the labelled grass silage a distinction was made between the DM fraction (^{13}CDM), the cell wall fraction (^{13}CNDR) and the non-cell wall fraction (^{13}CNDS). Passage kinetics of markers after introduction into the ileum and abomasum were estimated using an age-independent single exponential model. Faecal and ileal marker excretions after ruminal pulse doses were fitted using a multicompartmental model. Within experiments fractional passage rates between markers did not differ when pulse dosed into the ileum. After introduction into the abomasum differences became more pronounced but non-significant. A comparison between experiments showed that animals in Exp.2 had considerable lower fractional passage rates for all markers after both ileal and abomasal pulse doses. After a ruminal pulse dose, the ^{13}C labelled internal markers had distinctly lower fractional passage rates than those of Cr-NDF and Co-EDTA resulting amongst others in considerable higher total mean residence times (TMRT). In both experiments faecal and ileal determined TMRT's for ^{13}CNDR were the highest with values ranging between 59.5 h (ileal TMRT, Exp.1) and 94.5 h (ruminal TMRT, Exp.2). These values were approximately twice as high as those found for Cr-NDF with respective TMRT values of 30.1 and 41.1 h. Differences in functional specific gravity between labelled grass silage and Cr-NDF and the fact that the labelled material has to undergo fermentation to reach a certain functional specific gravity to escape the rumen, contribute to the explanation of the differences. Data suggests that ^{13}C forms a potential tool to study the relationship between passage and fermentation.

Key words: Passage rate, Grass silage, ^{13}C-isotope, Dairy cow

Introduction

In modern protein evaluation system for ruminants, metabolizable protein supply of individual animals are described by a microbial component, a bypass feed protein component, a correction factor for endogenous losses and the digestibility of these components in the small intestine (Vérité *et al.*, 1987; AFRC, 1992; Tamminga *et al.*, 1994). The proportions of

the bypass products depend on their feed specific fractional degradation rates and fractional passage rates (Robinson *et al.*, 1986). Within the Dutch system fixed values for fractional passage rates were adopted for roughages (0.045/h) and concentrates (0.060/h) (Tamminga *et al.*, 1994).

It is increasingly realized that passage rates also vary and depend among others on roughage treatment and quality (Bruining and Bosch, 1991; Rinne *et al.*, 1997; Kokkonen *et al.*, 2000), roughage type (Huhtanen and Hristov, 2001), diet composition (Yang *et al.*, 2001) and level of feed intake (Tamminga *et al.*, 1989; Colucci *et al.*, 1990). Moreover, recent developments in feed evaluation move towards dynamic nutrient based systems. In such dynamic systems, fractional passage rates form one of the most important factors as they determine the level and site of degradation and the efficiency of microbial protein synthesis (Dijkstra and France, 1996). Hence, for future development of nutrient based feed evaluation systems, knowledge on feed specific fractional passage rates is essential and up to this moment still lacking.

In this study the stable isotope of carbon, ^{13}C, was used as an internal marker to assess the feed specific passage kinetics of grass silage. Our objective was to create ^{13}C-labelled grass silage, study its passage characteristics through different compartments of the gastro-intestinal tract of dairy cows, compare its passage with that of Co-EDTA and Cr-NDF, and test the hypotheses that the passage dynamics of different markers differ from each other and that passage dynamics are affected by the level of feed intake.

Materials and Methods

In two subsequent experiments three cows were used to assess feed specific passage rates of feed particles originating from grass silage through different compartments of the gastro intestinal tract. To obtain information on feed specific passage rates the stable isotope of carbon, ^{13}C, was used as an internal marker. In addition, the *in situ* degradability of feedstuffs was determined in a separate experiment.

Marker Preparation

Small uniform and monospecific stands of rye grass (*Lolium perenne* L.) were selected in a pasture sown two years before. Two plots, each with an area of 2.15 m^2, were labelled with $^{13}CO_2$ at 10 different days during a regrowth period of six weeks. Upon harvesting, the labelled material was subjected to the same ensiling conditions as the bulk material. After a 6-week ensiling period, the labelled material was recovered from the bulk silage. The material was cut to an approximate length of 3 cm using a paper cutter, thoroughly homogenised and stored at –20°C pending further use. A representative sample was freeze-dried (FTS Dura-Dry programmable tray freeze drier), ground to pass a 1-mm screen (Wiley mill, T. Peppink & Zn., Machinefabriek, Amsterdam) and analysed for DM (ISO 6496) and neutral detergent

residue (NDR). The NDR was analysed according to a modified NDF-method of Van Soest *et al.* (1991) as described by Goelema *et al.* (1998) but omitting the ashing procedure. The non-cell wall fraction, i.e. the neutral detergent soluble fraction (NDS), was obtained by calculating the difference between the DM and NDR. The air-dried material and NDR were subsequently ground in a bullet mill (Retsch MM 2000) for 5 min at an amplitude of 80 Hz and analysed for total carbon content (TC) and ^{13}C-enrichment using an isotope ratio mass spectrometer (Finnigan_MAT CN). Enrichment results of the DM (^{13}CDM) and NDR ($^{13}CNDR$) are presented as delta ^{13}C ($\delta^{13}C$) or as atom percentage ^{13}C ($At\%^{13}C$). $\delta^{13}C$ expresses the ^{13}C:^{12}C ratio in a sample relative to the ^{13}C:^{12}C ratio of the international PDB standard (Pee Dee Belemnite; Eq.1) and $At\%^{13}C$ refers to the number of ^{13}C-atoms relative to the total count of C-atoms (Eq.2). The enrichment level of the neutral detergent solubles ($^{13}CNDS$) was derived from the difference in level of enrichment between the DM and NDR. The amounts of labelled grass silage required for pulse dosing into different segments of the gastro intestinal tract were determined on the basis of these enrichment results. Details of marker preparation are described by Pellikaan *et al.* (2004).

$$\delta^{13}C, \text{ in ‰ vs. PDB} = \frac{\left(^{13}C{:}^{12}C_{sample} - {}^{13}C{:}^{12}C_{standard}\right)\times 1000}{^{13}C{:}^{12}C_{standard}} \quad [1]$$

$$At\%^{13}C = \frac{\text{no. of atoms } ^{13}C}{\text{total no. of atoms C } (^{12}C,\ ^{13}C,\ ^{14}C)}\times 100 \quad [2]$$

In addition to the ^{13}C-labelled marker material, the solid phase external marker Cr mordant (Cr-NDF) and the liquid phase marker Co-EDTA were used. Both markers were prepared as described by Udén *et al.* (1980). With regard to Cr-NDF wheat straw was used as fiber source. After final drying procedure Cr-NDF was ground to pass a 0.5-mm screen.

Passage Experiments

Animals, housing and diets. Three first lactation Holstein-Friesian dairy cows fitted with rumen fistulae (Type 1C, Bar Diamond, Inc., Parma, Idaho, and U.S.A) and T-shaped ileum cannulae were used in two successive experiments. Ileal cannulae were constructed from silicone tubing with an i.d./o.d. of 25/34 mm. The experiments were carried out at the experimental farm “De Ossekampen” of Wageningen University where animals were housed in a tie-stall. During the first experimental period (Exp.1; Nov. 1997) animals were in early lactation, 57 DIM (SD = 21), had a BW of 484 kg (SD = 47) and produced 21.8 kg (SD = 0.7) fat and protein corrected milk/d (CVB, 1998). In the second experiment (Exp.2; May 1998) the animals were non-lactating and had an average BW of 501 kg (SD = 52). Surgery of animals to place rumen fistulae and ileal cannulae were conducted respectively, 10 to 15 weeks and 3 to 8 weeks before calving. Surgery procedures and experimental layout were

submitted to and approved of by an ethical committee, and executed in accordance with Dutch legislation on the use of experimental animals.

The experimental diets consisted of grass silage and a specially designed compound feed, fed in a roughage:concentrate ratio of 60:40 for DMI. For the composition of the compound feed ingredients were carefully selected to keep the level of naturally occurring ^{13}C-enrichment similar to that of the grass silage fed in both experiments (Table 1). In Exp.1 the animals were fed close to *ad libitum* feed intake, and to maintain the preset ratio between roughage and concentrate in individual diets feed residues were minimized. The total daily DMI was 12.3 kg/d (SD = 0.3) with 42.3% (SD = 0.5) of concentrate proportionally to the total DMI. In Exp.2 animals received about 60% (7.6; SD = 0.3 kg/d) of the individual DMI measured in Exp.1 with a concentrate proportion of 42.7% (SD = 0.5). Daily rations of

Table 1. Ingredients and chemical composition of the compound feed

Item	Compound feed[a]	Grass silage[b]
Ingredients		
Wheat	203.3	
Palm kernel expeller, crude fiber < 220	150.0	
Sunflower seed, extracted, crude fiber 160 – 200	26.3	
Soy hulls, crude fiber > 310	2.5	
Coconut expeller, crude fat < 100	150.0	
Beet pulp, sugars 100 – 150	400.0	
Vinasse, crude protein < 250	50.0	
Phosphoric acid limestone,	7.6	
Magnesium oxide, 100%	2.3	
Salt	3.0	
Minerals (Mervit rundvee 11)[c]	5.0	
Chemical composition		
DM, g/kg	900.0	391.0
OM	917.0	913.0
CP	148.0	172.0
DVE[d]	109.0	69.0
OEB[d]	-17.0	44.0
NDF	197.0	466.5
ADF	101.0	281.0
ADL	28.0	25.5
Starch	139.0	-
Sugars	79.0	95.0
NE_L, MJ/kg DM	7.2	6.0

[a] Chemical composition determined through linear programming, units given in g/kg of DM except when indicated otherwise.
[b] DM, OM, CP, DVE, OEB, sugars and NE_L determined through near infrared reflection spectroscopy (NIRS), Blgg, Oosterbeek, The Netherlands. NDF was analysed according to a modified method of Van Soest *et al.* (1991) as described by Goelema *et al.* (1998), ADF and ADL were analysed as described by Van Soest (1973).
[c] Mineral premix on basis of limestone, PRE-MERVO, Utrecht, The Netherlands.
[d] DVE = True protein digested in the small intestine, OEB = Degraded protein balance; units in the Dutch protein evaluation system as defined by Tamminga *et al.* (1994).

roughage and concentrate were divided into two equal portions and offered to the animals at 0630 and 1830 h. Orts were collected daily at 1800 h and weighed. Animals had free access to water and a commercially available mineral lick (KNZ®Rundvee, AKZO NOBEL SALT).

Treatments. Each measurement period was preceded by a two-week adaptation period for animals to get used to their diets. Subsequently, animals received pulse doses of ^{13}C-labelled grass silage, Cr-NDF and Co-EDTA in successively the ileum, abomasum and rumen. In case of pulse dosing into the ileum and abomasum, the labelled grass silage was first freeze dried and ground to pass a 1-mm screen (Retsch ZM1, centrifugal mill). Before introduction into the intestinal tract, the exact amounts of ^{13}C-labelled grass silage, Cr-NDF and Co-EDTA were soaked overnight in a 0.4% carboxy methyl cellulose solution (CMC); for the ileum and abomasum in respectively an 800 and 1200 ml CMC-solution. The marker containing solution was quantitatively placed into the ileum using a silicon tube (i.d. 8 mm) and squirt bottle. A silicon tube with similar diameter was fixed into the omasal-abomasal orifice via the rumen fistula. Subsequently, markers were introduced directly into the abomasum with a squirt bottle using gentle pressure. Before introducing the ^{13}C-labelled grass silage into the rumen the material was defrosted overnight in a refrigerator and acclimatised to ambient temperature. To administer the ruminal pulse doses the total rumen content was evacuated, mixed thoroughly with the markers and placed back quantitatively. Table 2 summarises the composition of pulse doses for individual animals.

Table 2. Composition of pulse doses for individual animals for different segments of the gastro intestinal tract during the two successive experimental periods

Dosing place	Co-EDTA[a] (g)	Cr-NDF[a] (g)	^{13}C-silage[b] (g)	^{13}CDM[c] (mg)	^{13}CNDR[c] (mg)	^{13}CNDS[c] (mg)
			Exp.1			
Ileum[d]	1.8	6.0	35.2	123.6	64.4	59.2
Abomasum[e]	3.0	10.0	50.6	177.4	92.4	85.0
Rumen	30.0	100.0	175.4	613.2	319.4	293.8
			Exp.2			
Ileum[d]	1.8	6.0	27.1	95.0	49.5	45.5
Abomasum[e]	3.0	10.0	45.2	158.4	82.5	75.9
Rumen	30.0	100.0	233.9	821.3	427.8	393.5

[a] Co-EDTA contained 148.5 g Co/kg ; Cr-NDF contained 39.8 g Cr/kg.
[b] Amounts given in g DM. DM content of labelled grass silage pulse dosed into the rumen 392.8 g/kg.
[c] Pulse dosed quantities of ^{13}C in mg for different fractions (DM, NDR and NDS) corrected for the naturally occurring amounts of ^{13}C.
[d] soaked in 800 ml 0.4% carboxy methyl cellulose solution.
[e] soaked in 1200 ml 0.4% carboxy methyl cellulose solution.

Sampling and chemical analyses. Roughage and concentrates were sampled each time portions for individual animals were weighed. Within experimental period samples were pooled. Orts of individual animals were sampled daily and pooled within an experimental period. Roughage and orts samples were dried at 60°C in a forced-air oven for 24 h and acclimatised to ambient conditions. Subsequently, samples were ground to pass a 1-mm screen (Wily mill) and analysed for DM, ash (ISO 5984), NDF, ADF and ADL. In addition, a roughage sample was analysed according to a standard NIRS-procedure (Table 1). Milk samples were collected during each milking and pooled per day. Samples were analysed for fat, protein and lactose using NIRS (Milk Control Station, Zutphen, The Netherlands).

Faecal samples were taken from each defecation after administration of the markers into the ileum for a 24-h period. Faecal samples were taken in a similar way for approximately 36 h after pulse dosing into the abomasum. Faecal sampling after ruminal pulse doses started 12 h after introducing the markers and was continued for about 110 h post treatment. From each defecation the faeces was quantitatively collected, weighed, homogenised and representatively sampled. Ileum chyme samples were only collected after the administration of markers into the rumen. Collection started 12 h after pulse dosing and during the first 24 h chyme samples were collected at 3-h intervals. Thereafter, chyme was collected at 6-h intervals, resulting in a total of 21 samples. The collected chyme was weighed, homogenised and sampled. Both faecal and chyme samples were directly stored at –20°C pending further analyses. Subsequently, samples were freeze-dried, ground to pass a 1-mm screen (Wiley mill) and analysed for DM, NDR, total carbon content (TC) in the DM and NDR and At%^{13}C in the DM (^{13}CDM) and NDR (^{13}CNDR). Cobalt and chromium were determined using an atomic absorption spectrophotometer (SpectrAA.300, Varian Inc., Palo Alto, VS).

Calculations and statistical analyses. The enrichment levels of the neutral detergent soluble fraction (^{13}CNDS) in the faeces and chyme samples were calculated from the difference in enrichment between the DM and NDR. The amount of ^{13}CDM and ^{13}CNDR was quantified from the total carbon content and corresponding At%^{13}C, after correcting for the change in molecular weight due to elevated levels of ^{13}C. The resulting quantity of ^{13}CNDS could be expressed relative to the calculated total carbon content of the NDS fraction (TC-NDS) after correction for molecular weight. By correcting the At%^{13}C of different fractions for the level of natural enrichment the faecal and ileal excretion patterns of the excess ^{13}C in the DM, NDR and NDS were established.

A single exponential equation (Eq.3) was fitted to the declining transect of faecal excretion curves of Co, Cr, ^{13}CDM, ^{13}CNDR and ^{13}CNDS following from pulse doses into the ileum and abomasum

$$Y\mathrm{t} = A \times \mathrm{e}^{(-K_p \times \mathrm{t})} \qquad [3]$$

where *Y*t expresses the marker concentration at time = t, *A* represents the calculated marker concentration at time = 0, K_p denotes the fractional passage rate constant, t is the average time

of defaecation, calculated as the mean value between two successive moments of defaecation. The average time was used to correct for differences in time spent by digesta in the distal part of the large intestine and rectum. The total mean retention time (TMRT) in these compartments was calculated from the reciprocal of K_p together with a correction for the time interval between the moment of marker introduction and the moment when peak concentration was reached (PCT). Faecal and ileal excretion patterns of markers following a pulse dose into the rumen were fit to a nonlinear multicompartmental model as proposed by Dhanoa *et al.* (1985) (Eq.4)

$$Yt = Ae^{-(K_1 \times t)} \exp\left[-(N-2)\, e^{-(K_2 - K_1) \times t}\right] \quad [4]$$

where Yt represents the faecal marker concentration at time = t, N denotes the number of compartments, K_1 and K_2 resemble the fractional rate constants for the two compartments in the digestive tract with the longest retention times and A forms a scalable parameter dependent on the N, K_1 and K_2. With regard to the collection of ileum chyme samples t was determined as the mean of the time span of collection post marker administration. From the parameter estimations on the basis of the multicompartmental model the transit time (TT, Eq.5), the total mean retention time (TMRT, Eq.6) and the moment of peak concentration (PCT, Eq.7) were calculated. Here, the TMRT is calculated from the reciprocal of the K_1 and K_2-values combined with the retention time associated with the remaining compartments, assumed to be equal to the TT. The TT represents the moment of first appearance of the marker in the faeces or chyme and the PCT resembles the moment when the excretion curve reaches peak concentration. Curve fitting was done using the nonlinear least squares regression procedure PROC NLIN (SAS Inst. Inc., Cary, NC). With regard to the parameter estimation for the Dhanoa model [Eq.4] a grid search of the full parameter space was conducted to obtain initial values for the iterative procedure. The array of initial values used were assessed from the excretion patterns and solved in 12 to 18 steps for each parameter. Faecal recovery (RP) of markers was calculated by analytical integration of the surface under the excretion curve and expressing the sum of these values relative to the amount of administered marker. A correction was made for marker losses due to ileal chyme sampling.

$$\text{TT, in h} = \sum_{i=3}^{N-1} 1 / K_2 + (\text{i} - 2) \times (K_2 - K_1) \quad [5]$$

$$\text{TMRT, in h} = 1 / K_1 + 1 / K_2 + \sum_{i=3}^{N-1} 1 / K_2 + (\text{i} - 2) \times (K_2 - K_1) \quad [6]$$

$$\text{PCT, in h} = \ln[(N-2) \times (K_2 + K_1) / K_2] / (K_2 - K_1) \quad [7]$$

Parameter estimates obtained from equations [3], [4] and the calculated TT, TMRT, PCT and recovery percentage were tested using the GLM procedure of SAS. Within the model, markers (Co, Cr, ^{13}CDM, ^{13}CNDR, ^{13}CNDS) were tested using Eq.8

$$Y_{ijk} = \mu + E_i + C_j + M_k + (E \times M)_{ik} + e_{ijk} \quad [8]$$

where Y_{ijk} represents the dependent variable, μ represents the mean, E_i corrects for the experimental period effect (i = 1, 2), C_j corrects for the animal effect (j = 1 - 3), M_k corrects for marker effect (k = 1 - 5), $(E \times M)_{ik}$ refers to the interaction, and e_{ijk} denotes the error term. The remaining two-way interaction terms did not significantly contribute to the model and hence, were considered to be part of the residual variance.

In Situ Experiment

Animals, housing and diet. Three mid-lactation Holstein-Friesian dairy cows fitted with a rumen fistula (Type 1C, Bar Diamond, Inc., Parma, Idaho, and U.S.A) and T-shaped ileum cannulas were used to assess the rate of fermentative degradation of feedstuffs fed to the animals in Exp.1 and 2. At the start of the experiment animals were 206 DIM (SD = 9), had a BW of 562 kg (SD = 25) and produced 22.0 kg (SD = 3.0) fat and protein corrected milk/d. The animals received grass silage and a commercially available standard pellet in a DMI ratio of 60:40. The feed was offered close to *ad libitum*, however, to ensure a 60:40 ratio between roughage and concentrate feed residues were set at a maximum of 5 to 10% of DMI. The total daily DMI during the experiment averaged 18.5 kg (SD = 1.8) with a concentrate proportion of 40.1% (SD = 0.5). Daily rations of roughage and concentrate were divided into two equal portions and offered to the animals at 0800 and 2000 h. Orts of individual animals were collected daily at 0800 h and weighed. Water and mineral lick were available at all times.

Treatments, sampling, chemical and statistical analyses. From the roughage and specially composed concentrate fed in Exp.1 and 2 samples were taken and stored at –20°C. The frozen roughage was cut to a size of 0.5 to 1.0 cm using a paper cutter, homogenised, split into several portions and stored at –20°C in airtight plastic bags. The compound feed was acclimatised, ground to pass a 3-mm screen, homogenised, placed in airtight plastic bags and stored at 4°C. After acclimatising to room temperature 15 × 8-cm nylon bags (pore size 40 µm, permeability 30%; PA 40/30, Nybolt, Switzerland) were filled with approximately 5 g of the test feeds on DM basis and randomly distributed over animals (3), incubation times (7) and series (12). Bags were always introduced into the rumen prior to the morning feeding and incubated for 2, 4, 8, 24, 48 and 336 h. Short term incubations (2 to 48 h) were done according to the all-in procedure. For each incubation cycle a maximum of 23 bags were placed in a larger-meshed nylon net connected to a stainless steel weight of 1.5 kg to ensure that bags remain incubated in the ventral part of the rumen sac. After adding the bags the net was closed and tied to the rumen cannula using a 70-cm nylon cord. Incubation was stopped by directly immersing the bags in ice water. The incubated bags were rinsed in tap water using gentle pressure, combined with bags containing the 0-h samples, placed in a domestic washing machine and washed in ambient tap water temperature for about 50 min., using ± 70 L of water (CVB, 1996). The washable fraction (W; 0 h) was determined from the loss of

weight in the 0-h bags relative to the amount of sample weighed in. In case the proportion of NDF-residue relative to the initial weight at t = 0 h was higher than 100%, W at t = 0 h was assumed zero. The residue after prolonged incubation for 336 h was determined using rumen fistulated animals that were housed in the free stall. The incubated bags received identical washing treatment. Bags were dried at 70°C in a forced-air oven for 24 h, and their contents was pooled by cow and incubation time and ground to pass a 1-mm screen (Retsch ZM1 centrifugal mill). Samples were analysed for DM, ash and NDF and data were fitted using an exponential equation as described by Robinson *et al.* (1986).

$$R\mathrm{t} = \mathrm{U} + (100 - \mathrm{U} - \mathrm{W}) \times \mathrm{e}^{(-K_d \times (\mathrm{t} - T))} \quad [9]$$

where Rt denotes the residue at time = t, U represents the undegradable fraction, W is the washable fraction, K_d expresses the fractional rate constant of degradation of the insoluble but potentially degradable fraction and T gives information on a possible lag time in onset of fermentative degradation. Parameter estimates obtained from Eq.9 were tested using PROC GLM. Within the model, degradation characteristics were tested using Eq.10.

$$Y_{\mathrm{ijk}} = \mu + F_{\mathrm{i}} + C_{\mathrm{j}} + N_{\mathrm{k}} + (F \times C)_{\mathrm{ij}} + (F \times N)_{\mathrm{ik}} + (C \times N)_{\mathrm{jk}} + e_{\mathrm{ijk}} \quad [10]$$

where Y_{ijk} represents the dependent variable, μ represents the mean, F_{i} corrects for the feed type effect (i = 1, 2), C_{j} corrects for the animal effect (j = 1 – 3), N_{k} corrects for nutrient type effect (k = 1 – 3) and e_{ijk} denotes the error term.

Results

Passage Experiments

Passage characteristics following marker introduction into the ileum and abomasum. Passage characteristics of the external (Co, Cr) and internal (^{13}CDM, ^{13}CNDR, ^{13}CNDS) markers after introduction into the ileum and abomasum at different levels of feed intake are summarised in Table 3. After an ileal pulse dose the fractional passage rates (K_p), the moment of peak concentration (PCT) and the total mean retention time (TMRT) showed no marker effect, but a significant animal effect. A comparison between experiments shows that the average K_p-value obtained at a high level of feed intake (0.416/h, Exp.1) was lower than that found in Exp.2 (0.281/h, $P < 0.001$). In combination with the PCT, a decrease of 40% in the level of feed intake resulted in an increase of the average TMRT in the large intestine with about 1.1 h; from 0756 (Exp.1) to 0901 (Exp.2) ($P = 0.004$). The recovery (RP) of external markers after ileal pulse dosing was complete with averages for Co and Cr of respectively 109.5 and 103.2%. The overall average RP for the internal markers (^{13}CDM, ^{13}CNDR,

^{13}CNDS) was clearly lower with respective values of 75.4, 79.6 and 74.8% ($P < 0.001$) indicating fermentation in the large intestine.

Introduction of markers into the abomasum showed significant marker effects for PCT, TMRT and RP within the model (Table 3). Although Co gave a higher K_p-value (0.267/h) than the other markers (0.134, 0.152, 0.183 and 0.165/h for Cr, ^{13}CDM, ^{13}CNDR and ^{13}CNDS) the differences were not significant ($P \geq 0.110$). During the second experiment one animal had a totally different excretion pattern with extreme low K_p-values and a very low RP compared to the other animals. At the moment of pulse dosing fixation of the abomasal infusion tube into the omasal-abomasal orifice gave problems, and hence, likely caused a backflow of the larger part of the marker containing solution into the omasal-ruminal cavity. Therefore, this animal was considered an outlier and consequently results were excluded from further statistical analyses. The overall animal effects were considerable for the PCT ($P < 0.001$) and to a lesser degree for K_p ($P = 0.027$). A comparison between experiments shows that the average K_p-value obtained at a high level of feed intake (0.263/h, Exp.1) differed from that obtained at a low level of feed intake (0.098/h, Exp.2) ($P = 0.001$). The average TMRT of markers in the post ruminal compartment increased for a low level of feed intake, from 1400 h in Exp.1 to 1934 h in Exp.2 ($P < 0.001$). The recovery (RP) of both Co and Cr was also complete after a pulse dose into the abomasum with averages of 111.3 and 99.1%. Like the average RP after ileal pulse doses, the average RP after abomasal pulse doses for the internal markers was also clearly lower with respective values for ^{13}CDM, ^{13}CNDR and ^{13}CNDS of 71.1, 79.0 and 60.7% ($P \leq 0.027$).

Passage characteristics following marker introduction into the rumen. Table 4 summarises the parameters assessed by the model (A, K_1, K_2, N) in combination with the coefficient of determination (R^2) for both experimental periods. Within the model, the fractional rate constant denoting the outflow from the slowest compartment (K_1) showed in general a significantly higher fractional passage rate of the liquid phase marker compared to the other markers ($0.021 < P < 0.001$), which is in line with expectations. The internal markers had considerable lower rate values for K_1 (0.029, 0.022 and 0.037/h for ^{13}CDM, ^{13}CNDR and ^{13}CNDS) when compared to those of the particle phase marker (Cr; 0.059/h), however, significant differences were not established ($P = 0.051$). The overall rate values estimated for the second slowest compartment (K_2) showed a similar pattern with significant higher fractional passage rates obtained for Co (0.469/h) compared to the other markers ($P < 0.008$) whilst differences between Cr (0.201/h) and ^{13}CDM, ^{13}CNDR and ^{13}CNDS (0.131, 0.111 and 0.165/h) were non significant ($P \geq 0.051$). The K_2-values of Co-EDTA determined from the ileal excretion curves were very high, 1.172/h in Exp.1 and 4.350/h for Exp.2 and were caused by the low number of data points before peak concentration was reached. With the exception of average N-value obtained from the faecal excretion pattern of ^{13}CNDS in Exp.1 (N = 166.8) all curve fits for individual markers gave values for N within a range of 5 to 75.

Table 3. Influence of level of feed intake and place of post-ruminal marker introduction on some passage characteristics[a,b]

	K_p (/h)			PCT (h)			TMRT (h)			RP (h)		
	Exp.1	Exp.2	(P>|T|)[e]	Exp.1	Exp.2	(P>|T|)[e]	Exp.1	Exp.2	(P>|T|)[e]	Exp.1	Exp.2	(P>|T|)[e]
	Ileal pulse dose											
Co	0.398	0.254	0.057	5.55	5.52	1.000	8.09	9.89	0.375	96.1^{wy}	122.8^{w}	0.006
Cr	0.422	0.266	0.032	5.50	5.47	1.000	7.89	9.81	0.296	102.1^{w}	104.2^{x}	1.000
^{13}CDM	0.429	0.302	0.122	5.49	5.42	1.000	7.83	9.14	0.757	75.4^{x}	75.3^{y}	1.000
^{13}CNDR	0.412	0.284	0.117	5.50	4.34	0.960	7.93	8.20	1.000	74.5^{x}	84.8^{xy}	0.732
^{13}CNDS	0.418	0.298	0.166	5.50	4.34	0.957	7.92	8.07	1.000	79.5^{xy}	70.1^{y}	0.818
SEM	0.029	0.029		0.67	0.67		0.53	0.53		4.1	4.1	
Model evaluation[d]												
rMSE		0.050			1.16			0.92			7.0	
Exp (I)		***			NS			**			*	
Cow (II)		***			†			***			*	
Marker (III)		NS			NS			NS			***	
(I × III)		NS			NS			NS			**	
	Abomasal pulse dose[c]											
Co	0.399	0.135	0.055	9.06	10.99^{wx}	0.294	11.97^{w}	17.94^{w}	0.001	101.5	121.0^{w}	0.417
Cr	0.198	0.071	0.739	10.54	12.77^{w}	0.165	15.61^{x}	22.71^{x}	0.000	96.2	102.0^{wx}	0.999
^{13}CDM	0.235	0.069	0.427	9.37	9.18^{x}	1.000	14.12^{wx}	19.19^{wx}	0.004	79.1	63.0^{yz}	0.650
^{13}CNDR	0.297	0.069	0.123	9.77	9.11^{x}	0.994	13.33^{wx}	19.17^{wx}	0.001	74.6	83.5^{xy}	0.980
^{13}CNDS	0.185	0.146	1.000	8.69	12.70^{w}	0.003	14.99^{wx}	18.81^{w}	0.033	75.1	46.2^{z}	0.081
SEM	0.044	0.056		0.46	0.58		0.59	0.75		5.2	6.5	
Model evaluation[d]												
rMSE		0.077			0.79			1.02			8.9	
Exp (I)		***			**			***			NS	
Cow (II)		*			***			***			**	
Marker (III)		NS			**			***			***	
(I × III)		NS			**			NS			**	

[a] K_p = fractional passage rate (/h) from the compartments; PCT = moment when excretion pattern reaches peak concentration (h); TMRT = (1/K_p) + PCT; RP = recovery percentage marker (%).
[b] Exp.1 = high level of feed intake; Exp.2 = low level of feed intake.
[c] Mean values for Exp.2 based on n = 2 animals; Abomasal infusion tube was not properly fixated in the omasal-abomasal orifice at the moment of marker introduction for one animal.
[d] Statistical model includes Exp (df2), Cow (df3), Marker (df5) as main effects and one two way interaction term; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.
[e] For comparison between experiments values for (P > |T|) indicates the level of significance.
[w, x, y, z] Within experiment and place of marker introduction (column) mean values differ ($P < 0.05$).

Table 4. Influence of level of feed intake and place of sample collection after marker introduction into the rumen on some passage parameters estimated using a multicompartmental model[a]

	Faecal excretion					Ileal excretion				
Item	A	K_1 (/h)	K_2 (/h)	N	R^2	A	K_1 (/h)	K_2 (/h)	N	R^2
	---------- Exp.1[b] ----------									
Co	10.50	0.099^{x}	0.569^{x}	31.3	0.947	8.18^{x}	0.154^{x}	1.172^{x}	53.7^{x}	0.972
Cr	9.23	0.069^{xy}	0.189^{y}	14.2	0.967	2.78^{y}	0.060^{y}	0.307^{y}	25.0^{xy}	0.942
^{13}CDM	0.06	0.034^{y}	0.147^{y}	16.4	0.968	0.02^{y}	0.033^{yz}	0.161^{y}	7.3^{y}	0.985
^{13}CNDR	0.03	0.028^{y}	0.134^{y}	18.6	0.979	0.02^{y}	0.026^{z}	0.136^{y}	9.5^{y}	0.965
^{13}CNDS	0.21	0.040^{y}	0.205^{y}	166.8	0.962	0.02^{y}	0.040^{yz}	0.171^{y}	6.8^{y}	0.969
SEM	4.79	0.011	0.057	56.4	0.009	0.62	0.006	0.066	7.7	0.011
	---------- Exp.2[b,c] ----------									
Co	35.32^{x}	0.104^{x}	0.368	71.5	0.991	7.33^{x}	0.114^{x}	4.350^{x}	9.1	0.986
Cr	7.66^{xy}	0.049^{xy}	0.214	74.2	0.995	4.60^{x}	0.053^{yz}	0.344^{y}	27.6	0.969
^{13}CDM	0.39^{y}	0.023^{y}	0.115	44.2	0.988	0.32^{y}	0.030^{yz}	0.148^{y}	11.6	0.971
^{13}CNDR	0.38^{y}	0.016^{y}	0.088	47.7	0.975	0.31^{y}	0.021^{y}	0.161^{y}	23.5	0.970
^{13}CNDS	0.42^{y}	0.035^{y}	0.126	44.4	0.986	0.43^{y}	0.064^{z}	0.167^{y}	12.5	0.976
SEM	6.06	0.013	0.072	71.3	0.011	0.79	0.008	0.084	9.7	0.014
Model evaluation[d]										
rMSE	8.30	0.018	0.098	97.6	0.015	1.08	0.011	0.115	13.3	0.019
Exp (I)	NS	NS	NS	NS	**	NS	NS	***	NS	NS
Cow (II)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Marker (III)	**	***	***	NS	NS	***	***	***	†	NS
(I × III)	NS	NS	NS	NS	NS	NS	**	***	*	NS

[a] K_1 = fractional passage rate (/h) from the slowest compartment; K_2 = fractional passage rate (/h) from the second slowest compartment; N = number of compartments; A = scalable parameter, dependant on the K_1, K_2 and N; R^2 = coefficient of determination.

[b] Exp.1 = high level of feed intake; Exp.2 = low level of feed intake.

[c] Mean values in Exp.2 based on n = 2 animals; One animal excluded due to excessive leakage of the ileal cannula following a pulse dose into the rumen.

[d] Statistical model includes Exp (df2), Cow (df3), Marker (df5) as main effects and one two way interaction term; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[x, y, z] Within experiment and place of sample collection mean values differ ($P < 0.05$).

Examination of the coefficient of determination (R^2) did not indicate differences in accuracy of the curve fitting between markers (results not shown). A comparison between experiments shows that the average K_1-value on the basis of faecal excretion at a high level of feed intake (0.054/h, Exp.1) was higher than at a low level of feed intake (0.045/h, Exp.2), but the difference was not significant (P = 0.317). Average K_1-values derived from ileal chyme samples showed a similar non-significant effect between experiments with averages of 0.063/h in Exp.1 and 0.056/h in Exp.2 (P = 0.221). When comparing the K_2 between experiments the average fractional passage rate based on faecal excretion decreases from 0.249/h (Exp.1) to 0.182/h (Exp.2) (P = 0.153). Ileal excretion patterns show an opposite and significant effect, however, this was solely explained by the extreme high K_2-values for Co-

EDTA. When comparing the markers with exclusion of Co-EDTA the effect for K_2 between experiments is in analogy with that obtained with the faecal excretion.

Compartmental retention times (CMRT1, CMRT2, TMRT), the transit time (TT) and the moment when markers reach peak concentration (PCT) are given in Table 5. Here the CMRT1 represents the mean retention time for slowest compartment ($1/K_1$), CMRT2 resembles that of the second slowest compartment ($1/K_2$) and TMRT equals the total mean retention time of both the CMRT1 and CMRT2 combined with the TT. From these data it appears that the internal markers (^{13}CDM, ^{13}CNDR, ^{13}CNDS) have a considerably longer TMRT than the external markers in all situations. With respect to the overall TMRT-values based on faecal excretion within the model, the cell wall marker ^{13}CNDR (82.6 h) gave a two-fold increase in TMRT compared to the particulate marker Cr (39.6 h) ($P < 0.001$). In addition also the moment of faecally determined PCT of ^{13}CNDR (47.0 h) was delayed by 20.0 h compared to that of Cr (27.2 h) ($P < 0.001$). Comparisons between experiments showed a delay in the moment of PCT due to a decrease in feed intake, but the effect was only significant for the cell wall marker ^{13}CNDR (40.1 h, Exp.1 vs. 53.8 h, Exp.2; $P = 0.004$). The TMRT of ^{13}CNDR on the basis of faecal excretion increased with 30 h, from 67.4 h to 97.9 h post dosing time ($P = 0.153$). On the basis of the ileal excretion the TMRT of ^{13}CNDR in Exp.2 gave an increase of about 17 h compared to Exp.1 ($P = 0.005$). The average recovery of Co and Cr in faecal and chyme samples was complete in Exp.2 with respective RP-values of 95.9 and 99.5% and slightly lower in Exp.1 with 82.6% for Co and 88.6% for Cr. With the exception of ^{13}CNDS in Exp.1 the RP of the internal markers appeared to be distinctly lower compared to the external markers. With regard to the DM fraction 43.4% of the ^{13}CDM was recovered in Exp.1 and for Exp.2 this was even lower (24.7%). Within experiments the RP of ^{13}CNDR tended to even lower values than that of the ^{13}CDM, however, differences were not significant ($P \geq 0.521$).

In Situ Experiment

Evaluation of the fractional degradation rate (K_d) and the lag time (T) shows significant effects of feed type (grass silage, compound feed), nutrient type (DM, OM, NDF), animal effect and their interaction terms within the model (Table 6). Within feed type NDF gave higher T-values than those of the DM and OM ($P \leq 0.002$), which is in line with expectations. The average T-value for the compound feed (57 min) was higher than that of the grass silage (32 min; $P < 0.001$). The cow effect on the T-value could be explained by one animal that had on average 0.5 h shorter lag time than the other animals. The overall average K_d-value of grass silage (0.027/h) was significantly lower than that for the compound feed (0.050/h). Within feed type the NDF fraction had a slightly lower K_d-value, however, only for the grass silage this effect was significant ($P < 0.015$). A comparison between animals showed that one animal had a significantly higher K_d-value than the other two (0.044 vs. 0.037 and 0.036/h; $P \leq 0.012$). Examination of data showed that the animal giving the higher K_d-value was not the same as the one with the shortest lag time.

Table 5. Influence of level of feed intake and place of sample collection after marker introduction into the rumen on compartmental retention times (CMRT1, CMRT2, TMRT), transit time (TT), moment of peak concentration (PCT) and marker recovery (RP)[a]

Item	Faecal excretion						Ileal excretion				
	CMRT1 (h)	CMRT2 (h)	TT (h)	TMRT (h)	PCT (h)	RP (%)	CMRT1 (h)	CMRT2 (h)	TT (h)	TMRT (h)	PCT (h)
	---------- Exp.1[b] ----------										
Co	10.15	1.93^{w}	6.56^{w}	18.64^{w}	11.18^{w}	82.6^{wx}	6.52^{w}	0.89^{w}	3.31^{w}	10.71^{w}	5.71^{w}
Cr	14.63	5.33^{wx}	15.51^{wx}	35.48^{wx}	25.66^{x}	88.6^{w}	17.01^{wy}	3.26^{wy}	9.87^{xy}	30.14^{x}	17.73^{x}
^{13}CDM	42.06	7.28^{wx}	15.71^{wx}	65.04^{x}	31.57^{xy}	43.5^{xy}	30.97^{x}	6.24^{xy}	9.52^{x}	46.73^{y}	23.72^{y}
^{13}CNDR	37.31	7.96^{x}	22.17^{x}	67.43^{x}	40.14^{y}	20.4^{y}	38.55^{x}	7.88^{x}	13.87^{y}	60.30^{z}	31.67^{z}
^{13}CNDS	41.08	6.19^{wx}	16.28^{wx}	63.55^{x}	28.24^{x}	68.6^{wz}	25.52^{xy}	5.91^{xy}	8.62^{x}	40.05^{xy}	21.22^{x}
SEM	8.34	1.04	2.14	6.14	1.62	7.5	2.14	0.63	0.78	2.04	1.17
	---------- Exp.2[b,c] ----------										
Co	11.23	2.54^{w}	12.42^{w}	26.19^{w}	18.32^{w}	95.9^{w}	8.81^{w}	0.29^{w}	0.55^{w}	9.66^{w}	1.25^{w}
Cr	21.25	4.37^{wx}	18.18^{wx}	43.80^{wy}	28.76^{wx}	99.5^{w}	19.52^{w}	3.43^{wx}	10.04^{x}	32.99^{x}	18.45^{x}
^{13}CDM	40.09	8.10^{wx}	20.13^{wx}	68.32^{xy}	39.30^{x}	23.0^{x}	33.87^{x}	8.58^{y}	12.64^{x}	55.08^{y}	30.19^{y}
^{13}CNDR	59.63	10.61^{x}	27.61^{x}	97.85^{x}	53.80^{y}	12.5^{x}	48.13^{y}	7.71^{y}	21.15^{y}	77.00^{z}	40.83^{z}
^{13}CNDS	29.05	7.45^{wx}	19.72^{wx}	56.23^{wy}	35.62^{x}	32.2^{x}	16.72^{w}	7.39^{y}	14.70^{x}	38.81^{x}	26.06^{x}
SEM	10.55	1.31	2.71	7.70	2.05	9.5	2.70	0.79	0.98	2.58	1.48
Model evaluation[d]											
rMSE	14.45	1.80	3.71	10.63	2.81	13.1	3.70	1.08	1.34	3.53	2.03
Exp (I)	NS	NS	*	†	***	NS	NS	NS	***	**	**
Cow (II)	NS	NS	NS	NS	*	†	NS	NS	**	NS	*
Marker (III)	**	***	***	***	***	***	***	***	***	***	***
(I × III)	NS	NS	NS	NS	NS	†	*	NS	***	**	**

[a] CMRT1 = 1 / K_1, retention time for the slowest compartment (h); CMRT2 = 1/ K_2, retention time for the second slowest compartment (h); TT = transit time (h), [Eq.5]; TMRT = CMRT1 + CMRT2 + TT (h), [Eq.6]; PCT = moment when summit of the excretion curve is reached (h), [Eq.7]; RP = marker recovery relative to the amount pulse dosed (%).

[b] Exp.1 = high level of feed intake; Exp.2 = low level of feed intake.

[c] Mean values in Exp.2 based on n = 2 animals; One animal excluded due to excessive leakage of the ileal cannula following a pulse dose into the rumen.

[d] Statistical model includes Exp (df2), Cow (df3), Marker (df5) as main effects and one two way interaction term; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[w, x, y, z] Within experiment and place of sample collection mean values differ ($P < 0.05$).

Table 6. *In situ* rumen degradation characteristics of grass silage and compound feed[a]

Item	W (%)	D (%)	U (%)	K_d (/h)	T (h)
Grass silage					
DM	26.3	58.8	15.0	0.0285[y]	0.34[y]
OM	22.6	62.4	15.0	0.0283[y]	0.45[y]
NDF	0.0	82.5	17.5	0.0259[z]	0.80[z]
Compound feed					
DM	40.1	53.8	6.1	0.0504	0.58[y]
OM	38.9	55.3	5.8	0.0498	0.56[y]
NDF	0.0	84.6	15.4	0.0495	1.72[z]
Model evaluation[b]					
SEM				0.0003	0.02
rMSE				0.0004	0.04
Feed type (I)				***	***
Nutrient (II)				**	***
Cow (III)				***	***
(I × II)				*	***
(I × III)				***	***
(II × III)				NS	*

[a] W = washable fraction; D = insoluble but potentially degradable fraction (100 – W – U); U = undegradable fraction; Fractions are expressed as percentage of the weighted sample; K_d = fractional degradation rate (/h); T = lag time (h).

[b] Statistical model includes feed type (df2), nutrient (df3), cow (df3) as main effects and three two-way interaction terms; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[y, z] indicate significant difference between nutrients (DM, OM, NDF) within feed type (grass silage vs. compound feed) ($P < 0.05$).

Discussion

Passage Through Different Compartments Following Post-Ruminal Marker Introduction

Faecally determined fractional passage rates of the external (Co, Cr) and internal (^{13}CDM, ^{13}CNDR, ^{13}CNDS) markers after introduction into the ileum showed no differences between markers (Table 3). These findings agree with the results of Huhtanen and Kukkonen (1995) and Mambrini and Peyraud (1997) who observed no differences between liquid and solid phase markers in the intestine following a pulse dose into the duodenum. In our study, the average TMRT in the large intestine ranged from 0756 h in Exp.1 to 0901 h in Exp.2, and fractional passage rates were significantly higher at the high intake level compared with the low intake level. In a study with two Hereford steers, O'Connor *et al.* (1984) observed mean retention times ranging between animals from 0520 h to 1140 h for a liquid phase marker and from 0800 h to 1350 h for a particle phase marker. Differences between the two animals were substantial and more contrasting than those observed in the current study.

After introduction into the abomasum differences between the markers became more pronounced, but remained non-significant. In contrast to the observations of Wylie *et al.* (2000) differences between the liquid and particle phase markers in the current study were more pronounced, in particular with regard to the *Kp*-values. Post-omasal compartmental residence times ($CMRT_{PO}$; $1/K_p$) measured in Exp.1, show that Co (0231 h) was lower than those of the particle phase markers, which range from 0322 to 0525 h ($P \geq 0.143$). A comparison between Exp.1 (high feed intake) and Exp.2 (low feed intake) shows a considerable effect of feed intake level on the K_p, the $CMRT_{PO}$ and the TMRT. Retention times at low feed intake level were higher ($P < 0.001$), and fractional passage rates lower ($P < 0.001$), than at the high intake level. With respect to the post-duodenal digesta kinetics Huhtanen and Kukkonen (1995) reported similar results in a study with Friesian bulls. With a contrast in the diet of 50% of DMI they observed average passage rates for high (80 g DM/kg $LW^{0.75}$) and low (40 g DM/kg $LW^{0.75}$) feed intake levels of respectively 0.298 and 0.169/h. In our study a contrast of 40% in feed intake level resulted in a decrease in K_p from 0.263/h to 0.098/h for respectively high (120 g DM/kg $LW^{0.75}$) and low (70 g DM/kg $LW^{0.75}$) intake diets.

When correcting the TMRT values obtained after pulse dosing into the abomasum with those found after pulse dosing into the ileum, three compartments and concomitant total mean retention times can be distinguished; a post-omasal compartment ($TMRT_{PO}$), a large intestine compartment ($TMRT_{LI}$) and derived from those the abomasal-small intestine compartment ($TMRT_{SI}$). The average $TMRT_{LI}$ of the three internal markers obtained in Exp.1 and 2 were respectively 0754 h (SD = 0004) and 0828 h (SD = 0035). For the $TMRT_{PO}$ respective retention times were found of 1409 h (SD = 0050) and 1814 h (SD = 0013), resulting in a $TMRT_{SI}$ of 0615 h (SD = 0050) in Exp.1 (1409 – 0754) and 1035 h (SD = 0029) in Exp.2 (1814 – 0828). Hence, a decrease in the level of feed intake results in an increase of the $TMRT_{SI}$ of about four hours and only an approximate 30-min increase for the $TMRT_{LI}$. This suggests that when animals are being subjected to a minor form of quantitative feed restriction the focus of post-ruminal digestion processes is more directed towards the small intestine compartment than to the large intestine compartment.

The quantitative recovery of the extra amount of ^{13}C, introduced into the ileum or abomasum was distinctly lower compared to that of Cr and Co suggesting a partial digestion (Table 3). The RP of ^{13}CDM after ileal pulse dosing showed no difference between Exp.1 (higher DMI, RP = 75.4%) and Exp.2 (lower DMI, RP = 75.3%). However, the RP of $^{13}CNDR$ increased with a decrease in DMI, whilst that of $^{13}CNDS$ decreased. After abomasal pulse dosing, the RP of ^{13}CDM and $^{13}CNDS$ were considerably lower in Exp.2 compared to Exp.1. The RP of $^{13}CNDR$ (84.8%) upon an ileal pulse dose in Exp.2 was higher compared to that of ^{13}CDM (75.3%) and $^{13}CNDS$ (70.1%). After an abomasal pulse dose these differences were even more profound with respective RP-values for $^{13}CNDR$, ^{13}CDM and $^{13}CNDS$ of 83.5, 63.0 and 46.2%. These data indicate that a decrease in the level of feed intake results in a decrease in post-omasal cell wall digestion, and preference is given to the apparent digestion

of the non-cell wall fraction. This matches with a shift towards an increased retention time in the small intestine compartment as discussed earlier. That the post-omasal compartment shows no preference to cell wall breakdown supports earlier work of Smith *et al.* (1967) who found little evidence of post-duodenal breakdown of ^{14}C-labelled NDF particles.

From these results it can be hypothesised that when ruminants are subjected to a moderate feed restriction, this results in an adaptation in the post-omasal digestive and fermentative processes. It is known that under such conditions rumen retention times increase (Huhtanen and Kukkonen, 1995), notably that of the cell wall fraction (the current study) and that ruminal fermentation processes alter (Robinson *et al.*, 1987). Besides, total tract apparent digestibility increases (Robinson *et al.*, 1985, Mulligan *et al.*, 2001), the cause of which is usually thought to be in the reticulo-rumen. However, the introduction of ^{13}C-labelled grass silage into the abomasum and ileum gives an indication that also adaptations occur in the post-ruminal compartments of the digestive tract.

Passage Through Different Compartments Following a Ruminal Marker Introduction

A deterministic multicompartmental model proposed by Dhanoa *et al.* (1985) was used to evaluate marker excretion patterns upon introduction into the rumen. The model characterises the gastro-intestinal tract by an unspecified number of exponential compartments in which behavior of the marker concentration is largely determined by the two slowest compartments, respectively the rumen and caecum. This multicompartmental model was preferred to other models because of its deterministic approach, the provision of two rate constants that relate to the compartments with the longest retention time, the possibility to establish multiple compartments ($N > 2$) within the gastro-intestinal tract and the robustness in fitting excretion patterns as compared to earlier models (Dhanoa *et al.* 1985; our unpublished observations). Stochastic models (Matis *et al.*, 1972, Pond *et al.* 1988) assume a physical limit of one age-independent and one age-dependent compartment. Observations of Pond *et al.* (1988) show that the mean residence time of the slower, age-independent compartment estimated from faecal excretion patterns agrees with excretion patterns found in duodenal digesta, hence, reflecting the reticulo-rumen. The age-dependent, faster turnover compartment appears to be related to both pre and post duodenal factors that determine a single fractional rate constant for this compartment. In this compartment, the slow imperfect mixing of particulate matter and the mixing abilities of hindgut compartments are non-discriminatory confounded.

In the work of Dhanoa *et al.* (1985) reference is made to two studies where roughage fed cattle received pulse doses of chromium oxide impregnated paper and Cr-mordant. For these markers fractional rate constants for K_1 and K_2 were obtained from faecal excretion patterns of respectively 0.042 and 0.026/h (Cr_2O_3) and 0.202 and 0.179/h (Cr-mordant). The values for K_1 of Cr used in the current study (Table 4) tended to higher values what can be explained by a combination of factors, e.g. marker applied, differences in BW, genetic merit and level of feed intake. When comparing the rate constant in the second slowest compartment (K_2) our findings match with the observations of Dhanoa *et al.* (1985). In other studies stochastic

models are used with various orders of age-dependency in one or more mixing compartments and a mixing compartment from where particles are assumed to have an age-independent probability to escape (Pond *et al.*, 1988). On the basis of the latter model, Poore at al. (1991) reported in dairy cows fractional rate constants in the slowest compartment for rare earth-labelled grain of approximately 0.075/h on the basis of faecal excretion which is slightly higher than the Cr in our study. With an average DMI of 23.6 kg/d versus a DMI of 12.3 kg/d (Exp.1) and 7.6 kg/d (Exp.2) the difference in fractional passage rate can be explained. Passage rates in dairy cows for the slowest compartment reported by Wylie *et al.* (2000) are more in line with the K_1-values obtained for the internal markers used in the current experiment. The Holstein Friesian heifers (475 kg BW) used in their experiment had a DMI of 9.5 kg/d which is comparable to that measured in our experiments. Huhtanen and Hristov (2001) used ^{15}N-labelled NDF and ADF as an internal feed specific marker for Lucerne silage and hay, pulse dosed it into the rumen of two late lactation dairy cows (DMI, 16.6 and 18.5 kg/d) and fitted duodenal and faecal excretion patterns with gamma age-dependent models with different orders of age dependency as described by Pond *et al.* (1988). Fractional rates representing the passage from the slowest compartment ranged between 0.030 and 0.053/h depending on animal, diet and order of age-dependency, and tended to slightly higher values than that of ^{13}CNDR in the current study. When comparing the faecally determined transit times (TT, Table 5) of the ^{13}CNDR in our study (22.2 – 27.6 h) with those in literature, the TT-values are distinctly higher than those reported by Huhtanen and Hristov (2001) who observed average values of 7.0 h for the ^{15}N-NDF labelled fraction. An important contributing factor to this difference in the TT may be the differences found in DMI of our animals (respectively 12.3 kg and 7.6 kg in Exp.1 and Exp.2) compared to those reported by Huhtanen and Hristov (16.9 kg). Another important factor is the type of model used to evaluate the excretion pattern. Mambrini and Peyraud (1994) evaluated several models (Grovum and Williams, 1973; Ellis *et al.*, 1979; Dhanoa *et al.*, 1985; Mambrini and Peyraud, 1994) in predicting faecal excretion patterns of europium-labelled forage and dysprosium-labelled concentrate. They concluded that the estimated moment of first occurrence of marker in the faeces (TT) was higher in the Dhanoa model than in the other models. Their observed TT-values for roughage (22.3 h) and concentrate (15.0 h) based on the model of Dhanoa, match to our findings and concomitantly explain the difference between our observations and the TT reported by Huhtanen and Hristov (2001) who used gamma age-dependent models. In general, faecally determined TT-values are believed to reflect the longitudinal digesta displacement through the more tubular segments of the intestinal tract. The time delay resulting from longitudinal passage through the large intestine (Table 3, PCT) determined after ileal pulse dosing averaged 4 to 6 hours, whilst the arithmetically determined residence time in the small intestine ($TMRT_{SI}$) averaged between 6.3 h (Exp.1) and 10.6 h (Exp.2). The faecal TT-values of the particle phase markers (Table 5) are considerably larger than the sum of the PCT and $TMRT_{SI}$. This indicates that TT-values derived from the model of Dhanoa do

not solely reflect the time delay caused by longitudinal passage through the intestines, and likely include a time delay that is related to pre-duodenal processes.

Comparing the total and compartmental mean retention times (Table 5) with the observations of Huhtanen and Hristov (2001) it appears that the retention times in the second slowest compartment CMRT2 are similar, whilst our CMRT1 and TMRT are substantially longer, especially with respect to those observed for ^{13}CNDR. However, Wylie *et al.* (2000) used time-dependent time-independent stochastic models and reported total mean retention times for masticated hay particles comparable to those observed in Exp.1. During the latter study the DMI was comparable with the levels of feed intake in our experiment, whilst the DMI reported by Huhtanen and Hristov (2001) was considerably higher. Thus, the smaller retention times at the higher intake level as compared to the low intake level in the present study, together with the qualitative comparison to other experiments that have a higher or comparable intake level, indicate that level of feed intake has a considerable effect on passage characteristics.

The higher K_1-values of ^{13}CNDS compared to those of ^{13}CNDR agree with the concept that cell wall constituents escape at a slower rate from the rumen compartment than the non-cell wall constituents. Our observations also showed that the K_1-values of ^{13}CNDS were always higher than those of ^{13}CDM. The faecal and ileal determined NDS-fraction comprises a complex matrix of apparent undigestible organic and inorganic components, originating from different sources (diet, microbes, animal). Assuming that both the microbes as well as the animals are fully adapted to the low ^{13}C-diet (C_3-plants), the addition of endogenous components in the digesta introduces a dilution factor for the ^{13}C:^{12}C-ratio. This could be one possible factor that increases the K_1 of ^{13}CNDS above that of the ^{13}CDM. The NDS fraction of roughage includes the non-cell wall associated constituents, which are to some extent more easily soluble in the liquid phase. Therefore, it can be hypothesised that the K_1 of ^{13}CNDS would arrive at a value between that of ^{13}CDM and Co. However, in most cases the K_1-values are even lower then those of Cr, suggesting that ^{13}CNDS is more representative for a non-cell wall fraction that is somehow associated with the fractional passage of the particle phase.

With respect to the compartmental residence times (Table 5), ^{13}CNDS gave a somewhat contradictory response in comparison to the other markers. Comparison between Exp.2 (lower intake) and Exp.1 (higher intake) showed that the TMRT of ^{13}CNDS was shorter due to a respectively 12-h (P = 0.994) and 9-h (P = 0.323) shorter faecal and ileal derived CMRT1. It was already mentioned that moderate levels of feed restriction causes an increase in rumen retention times (Huhtanen and Kukkonen, 1995) and alters ruminal fermentation processes (Robinson *et al.*, 1987). Earlier, we observed that due to a decrease in the level of feed intake preference is given to the post-omasal digestion of the non-cell wall fraction. This is likely related to an altered rumen functioning, where preference is given to cell wall degradation in favor of non-cell wall degradation.

The residence time of markers in the large intestinal-caecal compartment can alternatively be derived from the differences in time span at which TT and/ or PCT are reached for faecal and

ileal determined excretion patterns. Figure 1 visualizes these differences between faecal and ileal excretion patterns of Cr, ^{13}CDS and ^{13}CNDR. In Exp.1, the TT and PCT-values of ^{13}C-markers obtained from ileal excretion curves (Table 5) appear respectively 7.4 h (SD = 1.1) and 7.8 h (SD = 0.7) earlier compared to those obtained from faecal excretion curves, suggesting a large intestinal residence time of 7 to 8 hours. In Exp.2 differences between faecal and ileal TT and PCT-values are 6.3 h (SD = 1.2) and 10.6 h (SD = 2.1) respectively. In both experiments these values are comparable with TMRT-values obtained from faecal excretion patterns after direct pulse dosing into the ileum (Table 3). The CMRT2 estimated from faecal excretion curves using the multicompartmental model (Table 5) represents the markers residence time in the caecum. The average values for CMRT2 of the ^{13}C-markers obtained in our experiments (Exp.1, 7.1 h, SD = 0.9; Exp.2, 8.7 h, SD = 1.7) are comparable with those calculated from the faecal and ileal TT and PCT and those directly measured from the ileal pulse doses. This indicates that the multicompartmental model provides adequate information on large intestinal residence times that can be of further use in developing models describing the nutritional status of animals with the inclusion of a large intestinal compartment. One major advantage would be to have access to accurate information on the potential of digestive processes taking place in the hindgut estimated via a non-invasive technique.

Compared to the other markers ^{13}CNDR reaches the moment of PCT at a later stage and has lower K_1-values with subsequent longer retention times. This effect becomes even more pronounced in animals with low DMI (Exp.2). This suggests that the animal is able to

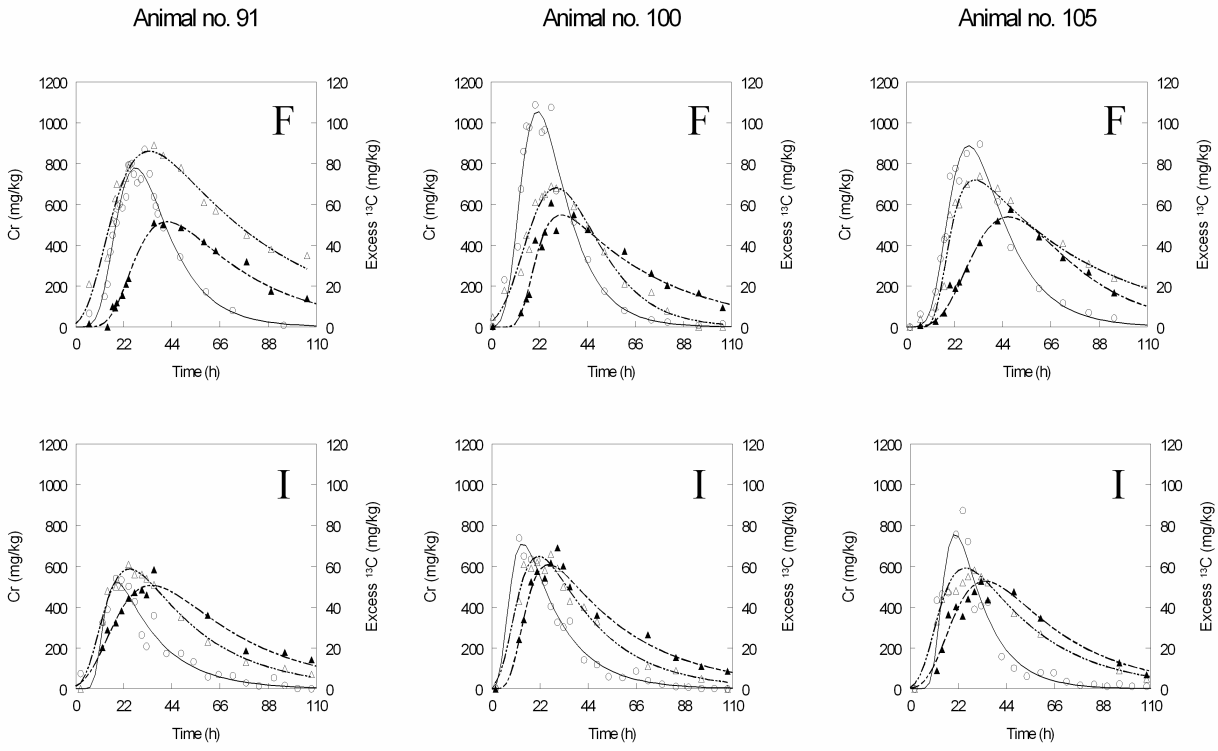

Figure 1. Faecal (**F**) and ileal (**I**) excretion patterns of Cr (○) and ^{13}C in the DM (Δ) and NDR (▲) after pulse dosing into the rumen for animals at a high level of feed intake (Exp.1), and the estimated excretion pattern for Cr (—— ○ ——), ^{13}CDM (-·-·- Δ -·-·-) and ^{13}CNDR (−··− ▲−··−) using a multicompartmental model

selectively retain this fraction for a longer period in the rumen before allowing it to escape from the rumen. This could be a possible explanation for the lower recovery of the ^{13}CNDR compared to the RP obtained for ^{13}CDM and ^{13}CNDS. However, when comparing the RP-values of NDR (Table 5) with the estimated amounts of rumen bypass NDF based on the equation ($U + D \times (K_1/(K_1 + K_d))$), bypass NDF would equal 60.4% (Exp.1) and 49.0% (Exp.2). This is considerably higher than the respective RP-values of NDR 20.4 and 12.5%. A small part of this difference is due to fermentation of a fraction of the rumen bypass NDF in the hindgut. The RP-values of the NDS, more specifically the one in Exp.1 (68.6%), are unexpectedly high compared to the estimated rumen bypass part of the NDF-fraction and the NDR recovery. This could also be explained by one of the animals (no. 91), which gave a near 100% recovery of ^{13}C in the NDS fraction. Exclusion of this animal reduces the RP to about 51%, which matches to the calculated amount of rumen by-pass NDS, based on OM degradation characteristics in combination with the faecally derived K_1-values for NDS.

A combination of the faecal and ileal determined RP-values gives information on the overall digestibility of the extra ^{13}C in the DM and NDR fraction. In Exp.1 ^{13}C digestibility coefficients of 56.7 and 79.6% were found for respectively the ^{13}CDM and ^{13}CNDR. In Exp.2 respective values of 77.0 and 87.5% were obtained. The low ^{13}CDM digestibility in Exp.1 could be partly explained by one of the animals (no. 91) that gave an RP of almost 60%. When examining the excretion pattern of this animal (Figure 1) it clearly differs from that of the other animals but the explaining causes remain unclear. Excluding this animal resulted in an increase in digestibility of approximately 10%, but remains low. The digestion of the ^{13}CNDR was quite high in both experiments, which was probably caused by the long TMRT for this marker. The actual balance trial data show similar differences between the apparent DM and NDR digestibility where NDR has about a 5 to 6%-units higher digestibility. The apparent DM and NDR digestibility in Exp.1 was 77.8 and 82.7% respectively and for Exp.2 73.3 and 79.1%. The underestimation of marker digestibility in Exp.1 was probably related to the partial recovery of the external markers, and corrected for this the digestibility for ^{13}CDM became 77.0% and that of ^{13}CNDR 92.9%. This data suggests an overestimation of the digestibility on the basis of ^{13}C compared to that obtained in the balance trials, however, more information is warranted.

Implications

The use of ^{13}C as an internal marker appears to be a promising tool in studying feed specific passage kinetics in ruminants. The work demonstrates that the external particle phase marker (Cr-NDF) overestimates the fractional passage rate constants, particularly with respect to that of the cell wall fraction. A decrease in the level of feed intake clearly resulted in decreased fractional passage rates and hence, increased compartmental and total tract residence times of markers. Prolonged residence time could be primarily explained by

increased ruminal residence time. The increase in post-ruminal residence time was primarily related to an increased residence time in the small intestinal compartment. Integration of the area under the excretion curves gives information on the ^{13}C recovery, i.e. digestibility of the ^{13}C-labelled feed components. As such, ^{13}C promises to be an interesting tool to investigate and quantify the *in vivo* relationship between fractional passage and degradation.

Acknowledgements

The authors kindly acknowledge the assistance of Mr. A. de Lange, Ms. A. Teunissen, Mr. D. Khot, Mr. H. van der Mast, Ms. M. Coppes, Ms. A. Dekker, Mr. K. Verburg, Mr. M. Los, Mr. A. van den Dool, Mr. L. Bijl, Ms. J-M. Muylaert, Mrs. M. van ‘t End and Ms. G. Post.

Literature Cited

AFRC. 1992. Nutritive requirements of ruminant animals: protein. AFRC Technical Committee on Responses to Nutrients. Report no. 9. Nutr. Abstr. Rev. (Series B). 62:787-835.

Bruining, M. and M.W. Bosch. 1991. Ruminal passage rate as affected by Cr-NDF particle size. In: Ph.D. Thesis of M.W. Bosch, Wageningen Agricultural University.

CVB, Centraal Veevoeder Bureau. 1998. Verkorte tabel 1998. Voedernormen landbouwhuisdieren en voederwaarde veevoeders. CVB-reeks nr. 24, Lelystad, The Netherlands.

CVB, Centraal Veevoeder Bureau, 1996. Voorlopig protocol voor *in situ* pensincubatie voor het meten van eiwitbestendigheid, 14 november 1996.

Colucci, P.E., G.K. McLeod, W.L. Grovum, I. McMillan and D.J. Barney. 1990. Digesta kinetics in sheep and cattle fed different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 73:2143-2156.

Dhanoa, M.S., R.C. Siddons, J. France and D.L. Gale. 1985. A multicompartmental model to describe marker excretion patterns in ruminant faeces. Br. J. Nutr. 53:663-671.

Dijkstra, J and J. France. 1996. A comparative evaluation of models of whole rumen function. Ann. Zootechnie., 45:175-192.

Goelema, J.O., M.A.M. Spreeuwenberg, G. Hof, A.F.B. van der Poel and S. Tamminga. 1998. Effect of pressure toasting on the rumen degradability and intestinal degradability of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Anim. Feed Sci. Technol. 76:35-50.

Huhtanen, P. and A.N. Hristov. 2001. Estimating passage kinetics using fibre-bound 15N as an internal marker. Anim. Feed Sci. Technol. 94:29-41.

Huhtanen, P. and U. Kukkonen. 1995. Comparison of methods, markers, sampling sites and models for estimating digesta passage kinetics in cattle fed at two levels of intake. Anim. Feed Sci. Technol. 52:141-158.

Kokkonen, T. M. Tuori and L. Syrjälä-Qvist. 2000. Effect of silage dry matter content and rapeseed meal supplementation on dairy cows 2. Rumen fermentation and digesta passage rate. Anim. Feed Sci. Technol. 84:229-242.

Mambrini, M., and J.L. Peyraud. 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet. Reprod. Nutr. Dev. 34:9-23.

Mambrini, M. and J.L. Peyraud. 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculations based on faecal collection. Reprod. Nutr. Dev. 37:427-442.

Mulligan, F. J., P. J. Caffrey, M. Rath, J. J. Callan, and F. P. O'-Mara. 2001. The relationship between feeding level, rumen particulate and fluid turnover rate and the digestibility of soya hulls in cattle and sheep (including a comparison of Cr-mordanted soya hulls and Cr2O3 as particulate markers in cattle). Livest. Prod. Sci. 70: 191-202.

O'Connor, J.D., P.H. Robinson, C.J. Sniffen and M.S. Allen. 1984. A gastro-intestinal tract simulation model of digesta flow in ruminants. Pages 102-121 in: Techniques in particle size analysis of feed and digesta in ruminants. Ed. P.M. Kennedy. Can. Soc. Anim. Sci. occasional publication 1, Edmonton, Alberta, Canada.

Pellikaan, W.F., H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven and S. Tamminga. 2004. ^{13}C-enrichment of cell wall fractions of rye grass (*Lolium perenne* L.) and implications for its use as digesta marker. Submitted as companion paper.

Poore, M.H., J.A. Moore, T.P. Eck and R.S. Swingle. 1991. Influence of passage model, sampling site, and marker dosing time on passage of rare earth-labeled grain through Holstein cows. J. Anim. Sci. 69:2646-2654.

Pond, K.R., W.C. Ellis, J.H. Matis, H.M. Ferreiro and J.D. Sutton. 1988. Compartment models for estimating attributes of digesta flow in cattle. Brit. J. Nutr. 60:571-595.

Rinne, M., P. Huhtanen and S. Jaakkola. 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol. 67:19-35.

Robinson, P.H., J.G. Fadel and S. Tamminga. 1986. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Anim. Feed Sci. Technol. 15:249-271.

Robinson, P. H., C. J. Sniffen, and P. J. van Soest. 1985. Influence of level of feed intake on digestion and bacterial yield in the forestomachs of dairy cattle. Can. J. Anim. Sci. 65: 437-444.

Robinson, P.H., S. Tamminga and A.M. Van Vuuren. 1987. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. Livest. Prod. Sci. 17:37-62.

Smith, L.W. 1989. A review of the use of intrinsically ^{14}C and rare earth-labeled neutral detergent fiber to estimate particle digestion and passage. J. Anim. Sci. 67:2123-2128.

Smith, L.W., D.R. Waldo, L.A. Moore, E.C. Leffel and P.J. Van Soest. 1967. Passage of plant cell wall constituents in the sheep. J. Dairy Sci. 50: 990 (Abstr.).

Südekum, K.H., W. Ziggers, N. Roos, H. Sick, S. Tamminga and M. Stangassinger. 1995. Estimating the passage of digesta in steers and wethers using the ratio of ^{13}C to ^{12}C and titanium(IV)-oxide. Isotopes Environ. Health Stud. 31:219-227.

Svejcar T.J, M.B. Judkins and T.W. Boutton. 1993. Technical note: labeling of forages with ^{13}C for nutrition and metabolism studies. J. Anim. Sci. 71:1320-1325.

Tamminga, S., P.H. Robinson, M. Vogt and H. Boer. 1989. Rumen ingesta kinetics of cell wall components in dairy cows. Anim. Feed Sci. Technol. 25:89-98.

Tamminga, S., W.M. Van Straalen, A.P.J. Subnel, R.G.M. Meijer, A. Steg, C.J.G. Wever and M.C. Blok. 1994. The Dutch protein evaluation system: the DVE/ OEB-system. Livest. Prod. Sci. 40:139-155

Udén, P., P.E. Colucci and P.J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31:625-632.

Van Soest, P.J. 1973. Collaborative study of acid-detergent fiber and lignin. J. Assoc. Offic. Anal. Chem. 56:781-784.

Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vérité, R., B. Michalet-Doreau, P. Chapoutot, J.L. Peyraud and C. Poncet. 1987. Révision du systeme des protéines digestibles dans l'intestin (PDI). Bulletin Technique C.R.Z.V. Theix, INRA. 70:19-34.

Wylie, M.J., W.C. Ellis, J.H. Matis, E.M. Bailey, W.D. James and D.E. Beever. 2000. The flow of forage particles and solutes through segments of the digestive tracts of cattle. Brit. J. Nutr. 83:295-306.

Yang, W.Z. K.A. Beauchemin and L.M. Rode. 2001. Barley processing, forage:concentrate, and forage length effects on chewing and digesta passage in lactating cows. J. Dairy Sci. 84:2709-2720.

Chapter 4

Passage of ^{13}C-Labelled Grass Through Different Compartments of the Gastro-Intestinal Tract of Dairy Cows

W.F. Pellikaan, J. Dijkstra, G. Hof[1], B.M. Tas, H. Boer, and S. Tamminga

[*]Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands

Submitted to British Journal of Nutrition

[1] in memory of G. Hof

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W.F. Pellikaan, J. Dijkstra, G. Hof, B.M. Tas, H. Boer, and S. Tamminga

Abstract

Feed specific passage rates of ^{13}C-labelled grass were determined in different compartments of the gastro-intestinal tract of dairy cows. Two rumen and ileum cannulated first lactation HF cows were pulse dosed with ^{13}C-labelled grass, Cr-NDF and Co-EDTA into the ileum, the abomasum and the rumen. The animals received a diet of grass and concentrate at a fixed ratio (75:25). Faecal marker excretion was determined after all pulse doses and ileal marker excretion was determined after a ruminal pulse dose. The labelled grass allowed differentiating between passage characteristics of the DM fraction (^{13}CDM), the cell wall fraction (^{13}CNDR) and the non-cell wall fraction (^{13}CNDS). Faecally determined passage rates upon an ileal pulse dose showed no differences between markers. After abomasal pulse dosing, differences became more pronounced but non-significant. Upon ruminal pulse dosing, the fractional passage rates of ^{13}CDM (0.025/h) and ^{13}CNDR (0.011/h) were lower (P<0.05) than those of Cr-NDF (0.041/h) and Co-EDTA (0.150/h). This resulted in considerable differences between the rumen related residence times, the total mean residence times, and moment when peak concentrations were reached. Feed intake patterns between animals differed considerably, but this did not seem to affect marker excretion patterns measurably. Fractional rumen clearance of DM (0.051/h) and NDF (0.034/h) as a function of the fractional rate constants of passage and digestion were in agreement with reports from rumen evacuations. It was concluded that ^{13}C is a valuable marker to characterize differences in passage kinetics between feed components.

Key words: ^{13}C-labelled grass, Marker passage, Isotope, Dairy cow

Introduction

International developments in feed evaluation for ruminants show a preference towards future use of systems of a more mechanistic and dynamic nature (Baldwin, 1995; AFRC, 1998; Dijkstra *et al.* 1998). In such systems, fractional passage rates form one of the most important factors as they determine the site and extent of degradation and the efficiency of microbial protein synthesis (Dijkstra and France, 1996). For future development of nutrient based feed evaluation systems, knowledge about feed specific retention times is essential information. Up to this moment such information is still largely lacking. Preliminary work of Svejcar *et al.* (1993) and Südekum *et al.* (1995) showed the potential of stable isotopes when used as internal markers. However, in their work fractional rates of passage were not quantified. Huhtanen and Hristov (2001) and Pellikaan *et al.* (2004[b]) used respectively ^{15}N and ^{13}C as internal markers to quantify passage kinetics originating from different roughage sources and feed fractions. Huhtanen and Hristov (2001) observed clear differences between the passage kinetics of ^{15}N incorporated within the NDF or ADF fractions of artificially labelled lucerne. In grass silage fed animals, Pellikaan *et al.* (2004[b]) observed that fractional

passage rates of ^{13}C in the DM and NDF differed considerably from Cr-mordant, and were affected by level of feed intake. Besides, the passage behaviour differed between the neutral detergent residue (NDR) and the neutral detergent soluble (NDS) fractions. Both studies showed that the use of stable isotopes as internal markers provide advantages to study passage kinetics compared to traditionally used external markers. Fractional passage rates are also affected by diet composition (Yang *et al.* 2001) and roughage quality (Bruining and Bosch, 1991; Rinne *et al.* 1997; Kokkonen *et al.* 2000), however, information on feed specific fractional passage rates are not available.

The main objective of the present study was to assess feed specific fractional passage rates of fresh grass through different compartments of the gastro intestinal tract of grass fed dairy cows using ^{13}C-labelled grass as internal marker. The traditionally used external markers Co-EDTA and Cr-NDF were included to compare and evaluate the behaviour of the internal marker.

Materials and methods

Marker Preparation

Small stands of a mixture of diploid and triploid rye grass (*Lolium perenne* L.) and timothy grass (*Phleum pratense* L.) were selected in a pasture sown two years before. Two plots, each with an area of 2.15 m^2, were labelled with $^{13}CO_2$ at four different days during a regrowth period of six weeks as described by Pellikaan *et al.* (2004[a]). One week after the final labelling treatment, at day 44 of the regrowth period, the plots were cut. The labelled grass was directly placed in an airtight plastic bag and stored at –20°C pending further treatment and analyses. The frozen material was cut to an approximate length of 3 cm, homogenised and restored at –20°C. A representative sample of this material was freeze-dried (FTS Dura-Dry programmable tray freeze drier), ground to pass a 1-mm screen (Wiley mill, T. Peppink & Zn., Machinefabriek, Amsterdam) and analysed for DM (ISO 6496), NDF and neutral detergent residue (NDR). The NDF was analysed according to a modified method of Van Soest *et al.* (1991) as described by Goelema *et al.* (1998), and the NDR was analysed as described by Pellikaan *et al.* (2004[a,b]). The non-cell wall fraction (NDS) was obtained from the difference between the DM and NDR. The air-dried material and NDR were ground in a bullet mill (Retsch MM 2000) for 5 min at 80 Hz and analysed for total carbon content (TC) and ^{13}C-enrichment using an isotope ratio mass spectrometer (Finnigan_MAT CN). Enrichment results of the DM (^{13}CDM) and NDR ($^{13}CNDR$) are presented as atom percentage ^{13}C (At%^{13}C), where the number of ^{13}C-atoms are scaled to the total count of C-atoms. The enrichment of the neutral detergent solubles ($^{13}CNDS$) was derived from the difference in ^{13}CDM and $^{13}CNDR$. In addition to the ^{13}C-labelled marker material Cr-mordanted wheat straw (Cr-NDF) and Co-EDTA were used. Both markers were prepared as described by Udén *et al.* (1980) and after drying Cr-NDF was ground to pass a 0.5-mm screen.

Passage Experiment

Animals and housing. Two first lactation Holstein-Friesian dairy cows (277 ± 5 DIM; mean ± SD) fitted with a rumen fistula (Type 1C, Bar Diamond, Inc., Parma, Idaho, U.S.A) and a T-shaped ileum cannula were used. The ileal cannulas were constructed from silicone tubing (i.d./o.d. 25/34 mm) and placed at 20 to 30 cm proximal to the ileo-caecal valve. Surgery of rumen fistulas and ileal cannulas were conducted at respectively 10 and 6 weeks before calving. The experiment was carried out at the experimental farm "De Ossekampen" of Wageningen University where animals were housed in a tie-stall. The stands were accommodated with automated feeding scales connected to a data logger (HP3852A, Data acquisition/ Control unit). Feed intake patterns of individual animals were monitored by recording weight readings every 30 sec, resulting in 2880 records per feeding scale per day. At the start of the experiment, animals had a BW of 512 ± 13 kg (mean ± SD) and produced 23.8 ± 2.9 kg (mean ± SD) FPCM/d. Handling of animals and experimental layout were submitted to and approved of by an ethical committee, and executed in accordance with Dutch legislation on the use of experimental animals.

Dietary treatment. The diet of the animals existed of defrosted grass that was harvested from the same pasture at the same day the labelled plots were harvested and was stored in large plastic bags at –20°C. This was done to ensure a constant grass quality in the diet throughout the experimental period. During the first two weeks of adaptation animals received grass by means of zero grazing. From the third week onwards animals were fed from the bulk of grass that was stored at –20°C. This material was allowed to defrost for one day prior to feeding. Daily rations of grass were divided into respective portions of 35, 30 and 35 kg fresh material and given to the animals at 0700, 1330 and 2000 h. In addition, animals received 4 kg of a specially designed compound feed that was divided into three equal portions and offered to the animals 15 min prior to the grass was given. The ingredients of the compound feed were carefully selected to keep the level of naturally occurring ^{13}C-enrichment similar to that of the grass in the diet (Table 1). The total daily DMI was 14.9 ± 1.0 kg/d (mean ± SD) with 24.3 ± 1.8% (mean ± SD) of concentrate proportionally to the total DMI. Orts were collected daily at 0700 h and weighed. Animals had free access to water and a commercial available mineral lick (KNZ®Rundvee, AKZO NOBEL SALT).

Treatments. Animals received pulse doses of ^{13}C-labelled grass, Cr-NDF and Co-EDTA in consecutively, the ileum, the abomasum and the rumen. Prior to pulse dosing into the ileum and abomasum, the frozen and cut labelled grass was further cut to an approximate size of 0.5 mm and allowed to acclimatise to ambient temperature for 24 h in sealed plastic bags. Twelve hours prior to introduction into the intestinal tract, the exact amounts of ^{13}C-labelled grass, Cr-NDF and Co-EDTA were soaked overnight in a 0.4% carboxy methyl cellulose solution (CMC, Fluka Chemie GmbH, Switzerland); for the ileum and abomasum in respectively an 800 and 1200 ml CMC-solution. The marker containing solution was quantitatively introduced into the ileum and abomasum using a 1.5-m long silicon tube (i.d. 25 mm) attached to a large plastic funnel. To place the tube into the abomasum, a part of the rumen

Table 1. Ingredients and chemical composition of the compound feed and the grass.

Item	Compound Feed*	Grass†
Ingredients		
Wheat	203.3	
Palm kernel expeller, crude fibre < 220	150.0	
Sunflower seed, extracted, crude fibre 160 – 200	26.3	
Soy hulls, crude fibre > 310	2.5	
Coconut expeller, crude fat < 100	150.0	
Beet pulp, sugars 100 – 150	400.0	
Vinasse, crude protein < 250	50.0	
Phosphoric acid limestone,	7.6	
Magnesium oxide, 100%	2.3	
Salt	3.0	
Minerals (Mervit rundvee 11)‡	5.0	
Chemical composition		
DM (g/kg)	900.0	132.0
OM	917.0	897.0
CP	148.0	172.0
DVE§	109.0	90.0
OEB§	-17.0	11.0
NDF	197.0	512.8
ADF	101.0	302.3
ADL	28.0	27.3
Starch	139.0	-
Sugars	79.0	115.0
NE_L, MJ/kg DM	7.2	6.0

* Chemical composition determined through linear programming, units given in g/kg of DM unless specified otherwise.
† DM, OM, CP, DVE, OEB, sugars and NE_L determined through near infrared reflection spectroscopy (NIRS), Blgg, Oosterbeek, The Netherlands. NDF was analysed according to a modified method of Van Soest *et al.* (1991) as described by Goelema *et al.* (1998), ADF and ADL were analysed as described by Van Soest (1973).
‡ Mineral premix on basis of limestone, PRE-MERVO, Utrecht, The Netherlands.
§ DVE = true protein available for digestion in the small intestine, OEB = degraded protein balance; units in the Dutch protein evaluation system as defined by Tamminga *et al.* (1994).

content was evacuated. Subsequently, the tube was fixed into the omasal-abomasal orifice by hand. Before introduction of the ^{13}C-labelled grass into the rumen, the material was defrosted overnight in a refrigerator and acclimatised to ambient temperature in sealed plastic buckets. Subsequently, the total rumen content was evacuated, mixed thoroughly with the three markers and placed back quantitatively into the rumen cavity. Table 2 summarises the composition of pulse doses for individual animals.

Sampling and chemical analyses. At the moment of harvest, representative samples were taken from the freshly cut grass. The compound feed was sampled each time portions for individual animals were weighed. Orts were sampled daily, dried at 60°C and pooled by animal. From the grass samples, sub samples were taken and pooled for a standard NIRS-

Table 2. Composition of pulse doses for individual animals for different segments of the gastro intestinal tract.

Dosing place	Co-EDTA* (g)	Cr-NDF* (g)	^{13}C-grass† (g)	^{13}CDM‡ (mg)	^{13}CNDR‡ (mg)	^{13}CNDS‡ (mg)
Ileum	1.8	6.0	41.9	105.4	58.3	47.1
Abomasum	3.0	10.0	59.6	182.9	101.1	81.8
Rumen	30.0	100.0	247.2	758.7	419.5	339.2
				---------- Isotopic enrichments§ ----------		
At%^{13}C labelled grass, %				1.7296	1.7102	1.7501
At%^{13}C unlabelled grass, %				1.0770	1.0776	1.0763

* Co-EDTA contained 135.9 g Co/kg; Cr-NDF contained 47.7 g Cr/kg.
† Amounts given in g DM. DM content of labelled grass 151.2 g/kg.
‡ Pulse dosed quantities of ^{13}C in mg for different fractions (DM, NDR and NDS) corrected for the naturally occurring amounts of ^{13}C.

analyses procedure (Table 1). The remainder of the grass samples were dried at 60°C, ground to pass a 1-mm screen (Wiley mill) and analysed for DM, ash (ISO 5984), NDF, ADF and ADL. In addition, representative feed samples were taken to be evaluated at a later stage for *in situ* degradation characteristics. Milk production and quality were determined by routine monthly milk testing where samples were analysed for fat and protein using NIRS (Milk Control Station, Zutphen, The Netherlands).

Faecal samples were taken at each moment of defecation starting directly after administration of the markers into the ileum and continued through to 100 h post ruminal pulse dose administration. Abomasal pulse dose was given 24 h after the ileal pulse dose and ruminal pulse dose followed at 30 h post abomasal pulse dose. From each defecation, the faeces was quantitatively collected, weighed, homogenised and representatively sampled. Ileum chyme samples were only collected after the administration of markers into the rumen. Chyme collection started 3 h after pulse dosing at three-h intervals during the first 24 h. Thereafter, chyme was collected at six-h intervals, resulting in a total of 22 samples. The collected chyme was weighed, homogenised and sampled. Faecal and chyme samples were stored at –20°C pending further analyses. Subsequently, samples were freeze-dried (FTS, Dura-Dry programmable tray freeze drier), ground to pass a 1-mm screen (Wiley mill) and analysed for DM, NDR, total C (TC) in the DM and NDR and At%^{13}C in the DM (^{13}CDM) and NDR (^{13}CNDR). The external markers were oxidized by wet-destruction at 350 °C for one hour in an HNO_3 (65%, 874380, Fluka Chemie GmbH) and $HClO_4$ (70-72%, 1005192500, Merck KgaA) solution and absorbency of Cr^{6+} and Co^{3+} was measured at respectively 357.8 and 251.0 nm in a nitrous oxide acetylene flow using an atomic absorption spectrophotometer (SpectrAA.300, Varian Inc., Palo Alto, VS).

Calculations and statistical analyses. Faecal and ileal excretion patterns of the excess ^{13}C in DM, NDR and NDS were established after correcting the At%^{13}C for natural enrichment. Faecal excretion curves of Co-EDTA, Cr-NDF and ^{13}CDM obtained after administration of

pulse doses into the ileum and abomasum were divided at peak concentration into two sections. The ascending and descending line sections were fitted using a single exponential equation as described by Pellikaan *et al.* (2004[b]), where the fractional rate constants for passage (*K*) were based on the descending section of the curve. The total mean retention times (TMRT) following ileal and abomasal pulse doses were calculated from the reciprocal of *K* (CMRT) together with a correction for the time interval between the moment of marker introduction and the moment when peak concentration was reached (PCT). PCT was derived by solving the estimates from ascending and descending line sections. Faecal and ileal excretion patterns of markers following a pulse dose into the rumen were fitted to a nonlinear multi-compartmental model proposed by Dhanoa *et al.* (1985), and the transit time (TT), the total mean retention time (TMRT) and the moment of peak concentration (PCT) were calculated from the model estimates as described earlier in detail in Pellikaan *et al.* (2004[b]). Curve fitting was done using the nonlinear least squares regression procedure PROC NLIN (SAS Inst. Inc., Cary, NC). With regard to the parameter estimation for the Dhanoa model a grid search of the full parameter space was conducted to obtain initial values for the iterative procedure. The array of initial values used were assessed from the excretion patterns and solved in 12 to 18 steps for each parameter. Recovery of markers (RP) was calculated by analytical integration of the surface under the faecal excretion curve and expressing the sum of these values relative to the amount of administered marker. A correction was made for marker losses due to ileal chyme sampling.

Parameter estimates and the calculated TT, TMRT, PCT and RP were tested using the GLM procedure of SAS. Within the model, markers (Co, Cr, ^{13}CDM, ^{13}CNDR, ^{13}CNDS) were tested using Eq.1

$$Y_{ij} = \mu + C_i + M_j + e_{ij} \quad [1]$$

where Y_{ij} represents the dependent variable, μ represents the mean, C_i corrects for the animal effect (i = 1 - 2), M_j corrects for marker effect (j = 1 - 5) and e_{ij} denotes the error term. Predicted faecal and ileal marker concentrations were compared with the observed values using the mean squared prediction error (MSPE; Bibby and Toutenberg, 1977) (Eq. 2)

$$MSPE = (\mu_P - \mu_O)^2 + (s_P - r \cdot s_O)^2 + s^2_O \cdot (1 - r^2) \quad [2]$$

where μ_O and μ_P represent the mean observed and predicted values with s^2_O and s^2_P as respective variances, and r^2 resembling the regression coefficient between the observed and predicted values. The root MSPE was scaled to the observed mean (Mean Prediction Error, MPE) and decomposed into errors due to overall bias ($\mu_O - \mu_P$; Bias), errors due to deviation of the regression slope from unity $(s_P - r \cdot s_O)^2$; Line) and errors due to random variation ($s^2_O \cdot (1 - r^2)$; Rand).

In Situ Experiment

Animals, housing and diet. Three first lactation Holstein-Friesian dairy cows (295 ± 8 DIM; mean ± SD) fitted with a rumen fistula (Type 1C, Bar Diamond, Inc., Parma, Idaho, U.S.A) were used to assess the rate of fermentative degradation of feedstuffs fed to the animals in the earlier described passage experiment. At the start of the experiment animals had a BW of 505 ± 11 kg (mean ± SD) and produced 22.3 ± 3.3 kg (mean ± SD) FPCM/d. Throughout the experimental period animals were allowed to graze freely in a pasture with perennial rye grass. In addition, animals received 4 kg of a commercially available standard pellet divided into two equal portions and given during the milking shifts at 0700 and 1630 h. Animals had free access to water and a commercial available mineral lick. All handlings of animals and experimental layout were submitted to and approved of by an ethical committee, and executed in accordance with Dutch legislation on the use of experimental animals.

Treatments, sampling, chemical and statistical analyses. Frozen samples of the grass that was offered to the cows during the passage experiment was cut to a size of 0.5 to 1.0 cm using a paper cutter, homogenised, split into several portions and stored at –20°C in airtight plastic bags. Nylon bags (15 × 8-cm, pore size 40 μm, permeability 30%; PA 40/30, Nybolt, Switzerland) were filled with approximately 5 g DM of the grass and randomly distributed over animals (3), incubation times (0, 2, 4, 8, 16, 24, 48 and 336 h) and periods (5). Incubations (2 to 336 h) were done according to the all-out procedure with a maximum of 25 bags per incubation cycle. Bags were retrieved from the rumen prior to the afternoon milking at 1500 h. Disappearance of sample from the bags with time was fitted using an exponential equation as described by Robinson *et al.* (1986), with inclusion of a lag time (*T*). Full details on the incubation procedure and statistical analysis are described by Pellikaan *et al.* (2004[b]).

Results

Passage Experiment

Feed intake pattern. Some characteristics of the feed intake patterns of individual animals are summarised in Table 3. The 2880 weight readings per feeding scale per day were separated into periods of eating (eating events) and periods of rest. Per feeding time (0700, 1330, 2000 h), successive eating events (n = 1 to 7) and corresponding time intervals were determined. To separate eating events the dataset was examined in two arithmetic steps. First, the advancing average weight values for 10-min intervals (20 successive recordings) were compared with every individual weight recording. At a difference of ≤ 40 g individual weight recordings were considered outlying values or indicating a resting (non-eating) period. In a second step, ten successive weight recordings were combined, creating 5-min intervals. In case the coefficients of variations (CV) for these intervals were 1 > CV > 35, the interval was considered an outlying values or was belonging to a resting period. Figure 1 visualizes the typical feed intake patterns (A) for both animals and the difference in intake behaviour

Table 3. The daily intake pattern, the intake pattern in relation to feeding times (0700, 1330, 2000) and the intake pattern in relation to eating events for individual animals.*

Item	n	GI (kg/d)	Eating time (h)	IR (kg DM/h)	IRf (/h)	n	GI (kg)	Eating time (h)	IR (kg DM/h)	IRf (/h)
	----------		Animal 61	----------		----------		Animal 65	----------	
Total	113	10.74 (1.11)	7.16 (0.07)	1.50	0.140	44	11.77 (0.58)	4.91 (0.08)	2.40	0.204
Averages per feeding time										
0700	33	3.88 (0.16)	2.57 (0.09)	1.51	0.141	15	4.24 (0.24)	1.79 (0.09)	2.37	0.201
1330	44	3.24 (0.11)	2.34 (0.06)	1.39	0.129	12	3.71 (0.17)	1.60 (0.07)	2.33	0.198
2000	36	3.61 (0.14)	2.25 (0.07)	1.61	0.149	17	3.81 (0.24)	1.52 (0.09)	2.51	0.214
Averages per eating event per feeding time										
1	24	2.30 (0.05)	1.25 (0.03)	1.84	0.171	26	3.46 (0.03)	1.37 (0.01)	2.52	0.214
2	26	0.79 (0.03)	0.62 (0.02)	1.28	0.119	12	0.27 (0.06)	0.22 (0.03)	1.23	0.104
3	21	0.20 (0.01)	0.19 (0.01)	1.03	0.096	4	0.22 (0.06)	0.17 (0.04)	1.32	0.112
4	20	0.16 (0.01)	0.17 (0.01)	0.95	0.088	2	0.03 (0.01)	0.12 (0.03)	0.28	0.024
5	15	0.11 (0.01)	0.16 (0.01)	0.68	0.063	-	-	-	-	-
6	5	0.22 (0.02)	0.24 (0.03)	0.89	0.083	-	-	-	-	-
7	2	0.10 (0.04)	0.12 (0.03)	0.81	0.075	-	-	-	-	-

* GI = grass intake (kg DM/d); IR = rate of roughage intake (kg DM/h); IRf = fractional rate of roughage intake relative to the total DMI (/h); Mean values ± SD between parenthesis.

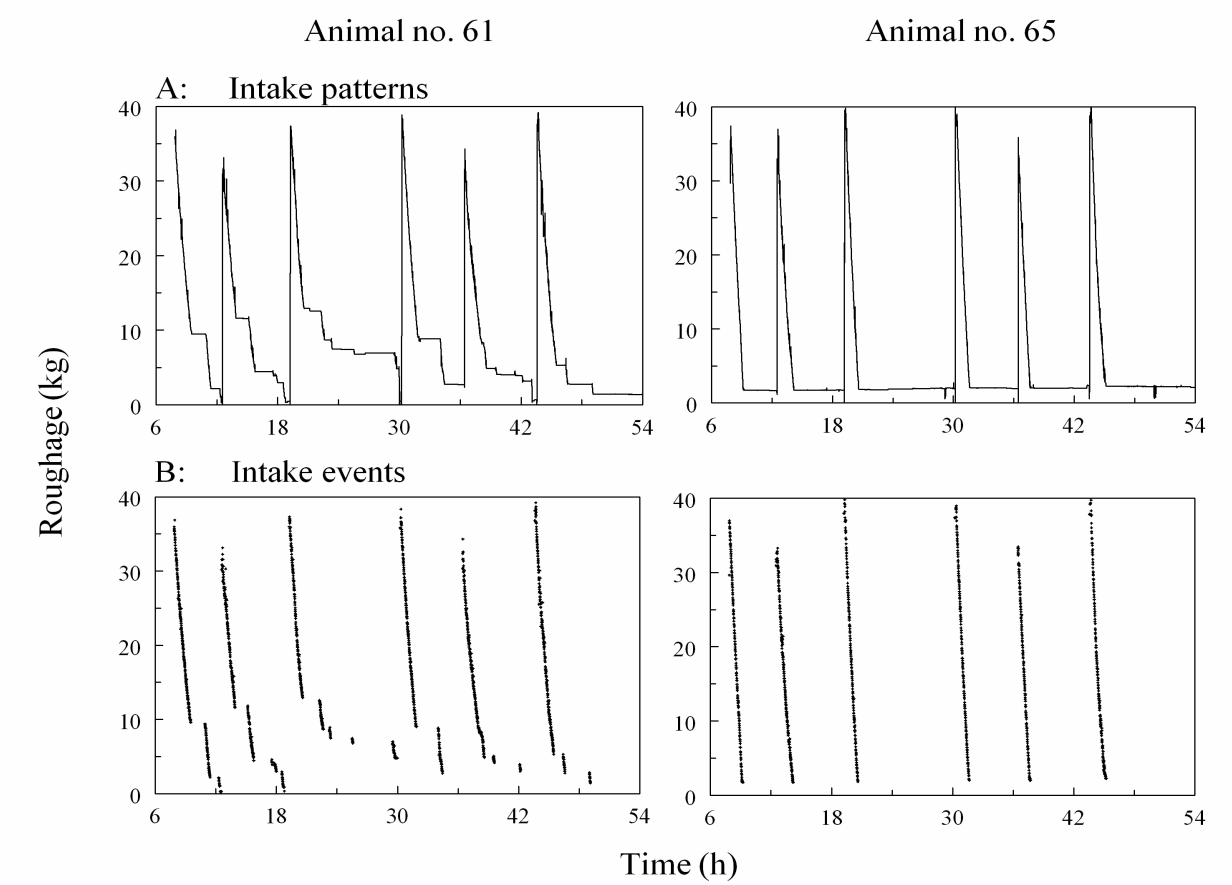

Figure 1. Typical intake patterns (A) and the associated intake events (B) of animal 61 and 65 during two successive d, where each peak in weight represents the time of feeding.

between them. The patterns corrected for non-eating periods reveal the individual intake events (Figure 1, B) from which the absolute and fractional intake rates were derived. During a 10-d period, a total number of 113 distinctive time intervals of active feed intake were recorded for animal 61 and 44 for animal 65 (Table 3). The average daily DM grass intake and the daily amount of time spent on eating were 10.7 kg in 7.16 h and 11.8 kg in 4.91 h for the respective animals, resulting in considerable higher intake rates for animal 65. In relation to the three feeding times (0700, 1330, 2000 h) the daily amount of time spent on eating was more or less equally distributed between the meals. It shows that animals concentrate their time to eat during the first event, directly after receiving the feed. Furthermore, it shows the difference in feed intake behaviour between animals. Animal 65 eats about 96% of its offered diet during the first event whilst animal 61 needs 3 to 4 events to reach this amount.

Marker passage following introduction into the ileum and abomasum. Passage characteristics of Co, Cr and ^{13}CDM after their introduction into the ileum and abomasum are given in Table 4. Upon ileal pulse dosing, the fractional passage rates (K), compartmental mean residence times (CMRT), and total mean residence times (TMRT) did not differ significantly between markers ($P \geq 0.274$). However, the moment when ^{13}CDM reached its peak concentration (PCT) gave a 20-min difference compared to the other markers ($P \leq 0.002$). The PCT after ileal pulse dosing differed significantly between animals ($P = 0.007$), whereas K, CMRT and TMRT were not affected by animal ($P \geq 0.452$). Marker introduction into the abomasum showed no differences in estimates of K, CMRT, PCT and TMRT between markers ($P \geq 0.331$). The effect of animal on K ($P \geq 0.103$), CMRT ($P \geq 0.166$), and TMRT ($P \geq 0.092$) after abomasal pulse dosing was in the same direction and more pronounced compared to those after an ileal pulse dose, but only for PCT ($P \geq 0.023$) the effect was significant. Marker recovery (RP) of Cr was complete whilst Co gave lower RP-values compared to Cr after introduction into the ileum ($P = 0.044$) and abomasum ($P = 0.128$). The RP-values of ^{13}CDM were distinctly lower then those from Co ($P = 0.056$) and Cr ($P = 0.022$), and some 50% of introduced label was not recovered.

The curve fits describing the declining part of the marker excretion patterns after ileal pulse dosing gave a model average for the root MSPE of 0.012 g/kg, resulting in an MPE of 23.2 ± 4.6% (mean ± SEM; data not shown). About 96% of the MSPE attributed to random disturbance. Between markers no differences in MPE were observed ($P \geq 0.926$), but differences in MPE between animal 61 (10%) and 65 (36%) were significant ($P = 0.040$), indicating that the excretion patterns of animal 65 were fitted with a lower accuracy. After abomasal pulse dosing, an average MPE of 17.7 ± 4.6% (mean ± SEM) was obtained, with no effects of markers ($P \geq 0.358$) and a non-significant difference between animals (animal 61, 12% vs. animal 65, 23%; $P = 0.187$).

Marker passage following introduction into the rumen. Faecally and ileally determined passage characteristics of Co, Cr, ^{13}CDM, ^{13}CNDR, and ^{13}CNDS after their introduction into the rumen are given in Table 5. Feacally determined fractional passage rates of Co for the slowest compartment (K_1) were higher than the other markers ($0.012 \geq P \leq 0.028$). Fractional

Table 4. Effect of marker and cow on faecally determined passage characteristics after post-ruminal (ileum, abomasum) marker introduction.[‡]

	Marker				Cow			Model evaluation[§]		
Item	Co	Cr	^{13}CDM	SEM	61	65	SEM	rMSE	Marker	Cow
Ileal pulse dose										
K, (/h)	0.359	0.329	0.404	0.042	0.347	0.381	0.035	0.060	NS	NS
CMRT, (h)	2.79	3.04	2.54	0.28	2.89	2.69	0.23	0.39	NS	NS
PCT, (h)	3.55^{a}	3.55^{a}	3.18^{b}	0.01	3.48^{a}	3.37^{b}	0.01	0.01	**	**
TMRT, (h)	6.34	6.58	5.72	0.28	6.36	6.06	0.23	0.39	NS	NS
RP, (%)	81.8^{a}	103.3^{b}	49.1^{c}	2.4	86.5^{a}	69.6^{b}	2.0	3.4	**	*
Abomasal pulse dose										
K, (/h)	0.360	0.267	0.247	0.042	0.223	0.360	0.034	0.059	NS	NS
CMRT, (h)	2.81	4.61	4.24	0.86	4.95	2.82	0.71	1.22	NS	NS
PCT, (h)	8.83	8.91	8.55	0.15	9.32^{a}	8.20^{b}	0.12	0.21	NS	*
TMRT, (h)	11.64	13.51	12.79	0.92	14.27	11.02	0.75	1.30	NS	†
RP, (%)	78.5^{ab}	97.5^{a}	48.5^{b}	3.8	87.2^{a}	62.5^{b}	3.1	5.4	*	*

[‡] *K* = fractional passage rate (/h) from the declining line; CMRT = 1/*K*, compartmental retention time (h); PCT = moment when summit of the excretion curve is reached (h); TMRT = CMRT + PCT; RP = marker recovery relative to the amount pulse dosed (%).

[§] Statistical model includes Marker (df3) and Cow (df2) as main effects; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[a,b,c,d] Within rows and main effects the LSmean values differ ($P < 0.05$)

Table 5. Effect of marker and cow on faecally and ileally determined passage characteristics after marker introduction into the rumen.[‡]

	Marker						Cow			Model evaluation[§]		
Item	Co	Cr	^{13}CDM	^{13}CNDR	^{13}CNDS	SEM	61	65	SEM	rMSE	Marker	Cow
Faecal excretion												
A	23.367	1.784	0.011	0.006	0.018	6.026	2.361	7.713	3.811	8.522	NS	NS
K_1, (/h)	0.150^{a}	0.040^{b}	0.025^{b}	0.011^{b}	0.035^{b}	0.015	0.042	0.062	0.009	0.021	*	NS
K_2, (/h)	0.467^{a}	0.311^{ab}	0.199^{b}	0.122^{b}	0.196^{b}	0.035	0.237	0.282	0.022	0.049	*	NS
N	33.5	47.9	17.3	11.6	15.5	11.9	19.9	30.4	7.5	16.9	NS	NS
CMRT1, (h)	7.07^{a}	24.74^{ab}	41.51^{b}	90.08^{c}	29.73^{ab}	4.54	44.12	33.13	2.87	6.42	**	†
CMRT2, (h)	2.15^{a}	3.44^{ab}	5.03^{bc}	8.44^{c}	5.11^{bc}	0.74	5.26	4.40	0.47	1.04	*	NS
TT, (h)	8.79^{a}	11.88^{ab}	12.82^{ab}	15.60^{b}	12.85^{ab}	0.72	12.49	12.28	0.46	1.02	*	NS
TMRT, (h)	18.00^{a}	40.06^{ab}	59.36^{b}	114.12^{c}	47.68^{b}	4.40	61.88^{a}	49.82^{b}	2.78	6.22	**	*
PCT, (h)	13.30^{a}	21.09^{b}	26.73^{b}	40.55^{c}	25.49^{b}	1.23	26.96	23.91	0.78	1.74	**	†
RP, (%)	75.0^{a}	72.2^{a}	19.9^{b}	36.2^{c}	44.7^{c}	1.9	48.2	51.0	1.2	2.7	***	NS
Ileal excretion												
A	2.242^{a}	1.296^{b}	0.012^{c}	0.005^{c}	0.017^{c}	0.129	0.669	0.759	0.082	0.182	***	NS
K_1, (/h)	0.100^{a}	0.044^{b}	0.034^{b}	0.009^{b}	0.045^{b}	0.007	0.043	0.050	0.005	0.010	**	NS
K_2, (/h)	4.536	2.218	0.250	0.363	0.213	0.792	1.820	1.212	0.501	1.120	†	NS
N	1.3E+6	1.3E+8	14.0	116.0	9.0	5.5E+7	1.3E+6	5.0E+7	3.5E+7	7.8E+7	NS	NS
CMRT1, (h)	10.25^{a}	23.33^{ab}	29.86^{b}	109.90^{c}	22.10^{ab}	2.37	39.87	38.30	1.50	3.34	***	NS
CMRT2, (h)	0.26^{a}	0.45^{a}	4.17^{b}	3.09^{ab}	4.82^{b}	0.52	3.07	2.05	0.33	0.74	**	†
TT, (h)	2.48^{a}	7.74^{b}	9.13^{b}	10.68^{b}	8.21^{b}	0.54	7.55	7.75	0.34	0.77	**	NS
TMRT, (h)	12.99^{a}	31.53^{b}	43.16^{c}	123.67^{d}	35.14^{bc}	1.76	50.50	48.10	1.11	2.49	***	NS
PCT, (h)	3.53^{a}	9.74^{ab}	20.02^{bc}	23.15^{c}	18.87^{bc}	1.78	15.98	14.15	1.13	2.52	**	NS

[‡] *A* = initial value of marker concentration; K_1 = fractional passage rate (/h) from the slowest compartment; K_2 = fractional passage rate (/h) from the second slowest compartment; *N* = number of compartments; $CMRT_1 = 1/K_1$ (h); $CMRT_2 = 1/K_2$ (h); TT = transit time (h); $TMRT = CMRT_1 + CMRT_2 + TT$ (h); PCT = moment when summit of the excretion curve is reached (h); RP = marker recovery relative to the amount pulse dosed (%).

[§] Statistical model includes Marker (df5) and Cow (df2) as main effects; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[a,b,c,d] Within rows and main effects the LSmean values differ ($P < 0.05$).

passage rates from the second slowest compartment (K_2) gave similar differences between Co and the other markers, but only in case of the internal markers (^{13}C-markers) differences were significant ($P \leq 0.025$). The fractional passage rates (K_1 and K_2) of ^{13}C-markers were in all cases lower than those for Cr ($P \geq 0.080$), with the lowest rate constants observed for ^{13}CNDR. Compared to the other markers, ^{13}CNDR had the highest values for CMRT1 ($P \leq 0.008$), CMRT2 ($0.018 \leq P \leq 0.137$), TT ($0.012 \leq P \leq 0.215$), TMRT ($P \leq 0.004$) and PCT ($P \leq 0.006$). The CMRT1, TMRT and PCT between ^{13}CDM and Cr differed considerable though non-significant with respective difference values of 16.8 h ($P = 0.229$), 19.3 h ($P = 0.148$) and 5.6 h ($P = 0.130$). Differences between the passage characteristics of ^{13}CNDS and Cr were similar to those observed between ^{13}CDM and Cr, but less pronounced. The lower number of estimated compartments (N) for ^{13}C-markers, as well as the lower estimates for A did not differ significantly from the external markers ($P \geq 0.204$). Comparison between animals indicates higher fractional passage rates for K_1 ($P = 0.204$) and K_2 ($P = 0.221$) in case of animal 65. Concomitantly, this animal had distinctly shorter values for CMRT1 ($P = 0.054$), TMRT ($P = 0.037$) and PCT ($P = 0.050$). Marker recovery (RP) of ^{13}CNDR and ^{13}CNDS was significantly higher then that of ^{13}CDM ($P \leq 0.019$) but all internal markers had lower RP-values compared to those of the external markers ($P < 0.003$). Although corrected for marker loss due to ileal sampling, the RP-values for Co and Cr were distinctly lower than the level of total marker recovery aimed at.

Excretion patterns based on ileum chyme samples depict similar relationships between markers, with highest estimates for Co in case of A ($P \leq 0.030$), K_1 ($P \leq 0.029$) and K_2 ($0.078 \leq P \leq 0.376$). Cr and Co gave extreme high values for K_2 and N, which is probably explained by the low number of observations ($n \leq 3$) before peak concentration was reached. Also here, ^{13}CNDR gave significantly longer CMRT1 and TMRT-values compared to the other markers ($P < 0.001$). The difference in PCT between ^{13}CDM and Cr became more pronounced then when faecally determined, however, the effect remained non-significant ($P = 0.066$). Compared to Cr, ^{13}CDM had a higher CMRT1 ($P = 0.418$), CMRT2 ($P = 0.032$) and TMRT ($P = 0.049$). Passage characteristics of ^{13}CNDS did not differ from those of Cr, with the largest effect observed for PCT ($P = 0.095$). No effects of animal were observed regarding the ileal determined passage characteristics.

Non linear model fits of the faecal marker excretion patterns were satisfactory with an average root MSPE of 0.007 g/kg. Scaled to the observed mean this resulted in an average MPE of 10%, with 94% of the MSPE attributed to random disturbance. With 13% the MPE for ^{13}CNDR was higher than the MPE for Co (6%, $P = 0.012$) and Cr (9%, $P = 0.085$), indicating less accurate model fits for the ^{13}CNDR excretion patterns. Animal effect did not contribute to the MPE ($P = 0.172$). Fits of the ileally determined marker excretion patterns resulted in an average root MSPE of 0.0135 g/kg or 15% of the observed mean (MPE), with 96% of the MSPE attributing to random disturbance. No effects of marker ($P \geq 0.981$) and animal ($P \geq 0.450$) were observed.

In Situ Experiment

In Table 6 the *in situ* degradation characteristics of different components of fresh grass are given. The crude protein fraction (CP) had the highest fractional rate constant for degradation (K_d) ($P \leq 0.001$) and NDF had the lowest ($P \leq 0.014$). The lag time (T) of the NDF fraction did not differ from zero ($P = 0.704$) but T-values of other nutrients did differ significantly from zero ($P \leq 0.025$). In all cases, the Cow effect contributed significantly to the model estimates ($P \leq 0.016$).

Discussion

Marker Passage after Post-Ruminal Marker Introduction

The fractional passage rate constants from the large intestine compartment (i.e. following from a pulse dose into the ileum) were within the range of rate constants reported by Pellikaan *et al.* (2004[b]). In an experiment with two Hereford steers, O'Connor *et al.* (1984) observed large differences in faecal excretion patterns between animals after ileal pulse dosing of Co-EDTA and ytterbium marked alfalfa, with large intestinal TMRT-values of 5.3 to 11.7 h for Co and 8.0 to 13.8 h for Yb. The TMRT of Co observed in the current study fell within this range, however, in case of Cr and ^{13}CDM TMRT-values were considerably lower. Factors like diet composition, daily DM intake, type of animal and type of marker were probably contributing to these differences. In contrast to observations in grass silage fed animals at two levels of intake (Pellikaan *et al.*, 2004[b]), in the current study the average moment of PCT (3.4 h) occurred about 2 h earlier relative to the moment of marker introduction and TMRT

Table 6. *In situ* rumen degradation characteristics of the grass.[†]

Item	W (%)	D (%)	U (%)	K_d (/h)	T (h)
DM	27.5	61.6	10.8	0.0264[a]	0.81[ab]
OM	22.7	66.3	11.0	0.0265[a]	0.91[ab]
NDF	0.0	85.3	14.8	0.0229[b]	0.11[a]
CP	33.4	59.3	7.3	0.0492[c]	1.77[b]
Model evaluation[‡]					
SEM				0.0005	0.27
rMSE				0.0009	0.47
Nutrient				***	*
Cow				**	*

[†] W = washable fraction; D = insoluble but potentially degradable fraction, 100 – W – U; U = undegradable fraction determined after 336 h of incubation; Fractions are expressed as percentage of the weighted sample; K_d = fractional degradation rate (/h); T = lag time (h).

[‡] Statistical model includes nutrient (df 4) and cow (df3); rMSE = root mean square error; * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[a,b] indicate significant difference between nutrients (DM, OM, NDF, CP) ($P < 0.05$).

averaged 6.2 h compared to 7.9 h and 9.0 h in cows kept at high and low levels of DMI, respectively. The average daily DMI (14.9 kg) of animals in the current study was approximately 2.5 kg higher than the grass silage fed animals kept at higher level of feed intake whilst the contrast with the low feed intake group was 7.3 kg of DMI (Pellikaan *et al.*, 2004[b]). This tendency of decreasing TMRT following ileal pulse dosing in relation to higher levels of DMI agrees with general observations in literature (Huhtanen and Kukkonen, 1995; Pond *et al.*, 1988 and Poore *et al.*, 1991 as discussed by Mambrini and Peyraud, 1997). However, information on lower tract residence times are mainly based on faecal excretion patterns following from duodenal (Huhtanen and Kukkonen, 1995; Mambrini and Peyraud, 1997) or abomasal marker administration (Wylie *et al.*, 2000), or by deriving the small and large intestinal residence times from the difference between faecal and duodenal excretion patterns following markers introduction into the rumen (Poore *et al.*, 1991; Pond *et al.*, 1988; Huhtanen and Hristov, 2001).

Similar to the absence of significant marker effects on residence times after introduction into the ileum, no significant differences were observed in *K*, CMRT, PCT and TMRT between markers after introduction into the abomasum, which agrees with literature (Huhtanen and Kukkonen 1995; Mambrini and Peyraud, 1997; Wylie *et al.*, 2000). However, observation of our excretion curves (not presented here) indicates differences in passage behaviour between liquid phase and particle phase markers, where especially Cr tends to 'trailing' at the descending part of the curve. This corresponds with earlier findings of Pellikaan *et al.* (2004[b]), and agrees with observations reported by O'Connor *et al.* (1984) who suggested that particle phase markers could be selectively retained in the caecal-large intestine compartment compared to liquid phase markers. However, observed differences between the *K*-values of Co and Cr (0.093/h) and Co and ^{13}CDM (0.113/h) presented in Table 4 were not significant ($P \geq 0.331$), and hence, do not substantiate the visual indication that passage behaviour of the liquids and solids tend to differ in the post-rumen compartments.

In line with expectations, the recovery (RP) of Cr was complete. In contrast, Co gave lower RP-values after an ileal pulse dose (81.8%; $P = 0.044$) and abomasal pulse dose (78.5%; $P = 0.128$). Part of this lower recovery can be contributed to loss of Co through uptake via the intestinal wall as reported in literature (Udén *et al.*, 1980). Corrected for this percentage (2 to 3% loss), differences in RP between Cr and Co remain considerable but non-significant. The RP-values for ^{13}CDM after an ileal (49.1%) and abomasal (48.5%) pulse dose suggest that a considerable amount was digested in the post-ruminal compartments. Moreover, the similarity of RP-values between pulse dose sites indicates that no substantial digestion ^{13}CDM occurs in the small intestine compartment. Pellikaan *et al.* (2004[b]) observed similar effects for ^{13}C-labelled DM in the small intestine in grass silage fed animals, but RP-values after ileal and abomasal pulse doses were considerably larger, averaging respectively 75 and 71%. The difference in roughage type, roughage quality, method of conservation (frozen vs. ensiled) and difference in treatment before pulse dosing (fresh and cut to 5 mm vs. freeze dried and ground to 1 mm) are possible factors contributing to the differences between the experiments.

However, the grass used in the current experiment had higher NDF, ADF and ADL values than the silage used by Pellikaan *et al.* (2004[b]), and *in situ* degradation characteristics (W, U and K_d) did not differ between experiments. Hence, roughage type and quality are unlikely to contribute to the 20 to 25% difference in RP between experiments. Therefore, the main contributing factor seems to be the difference in pulse dose treatment between experiments. This agrees with Boudon and Peyraud (2001) who observed that treatments like freeze drying, grinding and or mechanical cutting of fresh herbage affect the soluble (W) and degradable (D) fractions. The marker bypass part (i.e. RP) can also be calculated from the OM *in situ* degradation characteristics (Table 6) and the large intestinal TMRT (ileal TMRT, Table 4). This results in a considerably higher calculated RP-value for ^{13}CDM of 69%. Therefore, although some factors that contribute to the difference may be identified, it can be concluded that the RP measured in the current experiment is inexplicably low.

Marker Passage after Ruminal Marker Introduction

The faecally determined fractional passage rates for the slowest (K_1) and second slowest compartment (K_2) upon ruminal pulse dosing (Table 5) are comparable with those observed by Pellikaan *et al.* (2004[b]) in dairy cows assigned to higher (12.3 kg DM/d) and lower (7.6 kg DM/d) levels of feed intake. Interestingly, the K_1-values of Cr and ^{13}C-markers were more comparable to those of the animals assigned to low intake diet, whilst the animals in the current experiment had a considerable higher intake (14.9 kg DM/d). Thus, next to feeding level, feed quality also affects the fractional passage rate. Attributing factors could be difference in roughage quality and diet composition between the current and the aforementioned experiment. The current experimental diet included some 24% concentrates, which contrasted considerably with the level of concentrates included in the grass silage based diet (42% of total DMI). In general, a decrease in the amount of concentrate in the diet results in an increase of the fractional liquid outflow rate from the rumen (Owens & Goetsch, 1986; Colucci *et al.*, 1990). Indeed, the estimated fractional passage rate with Co was lower in the current experiment than in the grass silage experiment. The effect of concentrate level on particle passage rate is less clear. This effect might be slightly positive (Owens & Goetsch, 1986) or negative (Colucci *et al.* 1990). In addition, the grass used in the current experiment was high in NDF, ADF and ADL content (Table 1). Such effects are known to cause increased fractional rumen passage rates (Gasa *et al.* 1991; Rinne *et al.*, 1997). In contrast, Van Vuuren (1993) reasoned that the fractional passage rates of materials with higher NDF and lower degradation rates would be lower to ensure fermentation and protein availability. But, with a CP and DVE content of 172 and 90 g/kg respectively, protein availability in the rumen was unlikely to be the limiting factor.

^{13}C-marked fractions (DM, NDR, NDS) had lower K_1-values compared to Cr with lowest values for ^{13}CNDR and highest for ^{13}CNDS. The difference between NDR and NDS passage is likely related to the slower rumen escape rates of the cell wall components compared to those of the non-cell wall components (Pellikaan *et al.*, 2004[b]). Theoretically, the apparently

undigested ^{13}CNDS should to some extent represent the passage behaviour of the potentially degradable non-cell wall fraction. This fraction is to a large extent readily soluble in the liquid phase, hence, resulting in relatively higher fractional passage rates compared to ^{13}CNDR and ^{13}CDM. Pellikaan *et al.* (2004[b]) further hypothesised that the higher K_1-value for ^{13}CNDS compared to ^{13}CDM was partly related to endogenous secretions that cause extra dilution in the ^{13}C:^{12}C-ratio. Although ^{13}CNDS seems to generate rational information on passage rate constants, the exact interpretation of these data remains unclear and more information is warranted.

The estimates on the CMRT1, CMRT2, TT, TMRT and PCT of Co, Cr and ^{13}CDM as presented in Table 5 are comparable with observations of Pellikaan *et al.* (2004[b]). In a study with mid-lactation dairy cows (DMI, 17.7 kg/d), Mambrini and Peyraud (1997) observed faecally determined TMRT (45.6 h), CMRT1 (24.3 h) and TT (10.5 h) of Ytterbium-labelled ground hay with a mean particle size of 0.6 mm, that were similar to those of Cr in the current study. Their observed values for CMRT2 (10.9 h) was considerably longer than the CMRT2 of Cr (3.4 h) in this experiment. However, their CMRT2 was indirectly calculated from the TMRT minus the CMRT1 and TT, implying linearity in the time interval between TT and PCT, and independency between the ascending and descending parts of the excretion curve. It appeared that the differences between approaches (Dhanoa model vs. Mambrini and Peyraud method) seem to be primarily related to the difference in residence time based on the ascending part of the excretion curve. In the model of Dhanoa *et al.* (1985) the fractional rate constant (K_2) describing the ascending part of the line likely represents the caecal compartment. In contrast, Mambrini and Peyraud (1997) relate an important part of their observed CMRT2 to processes restricted to the rumen compartment (i.e., time spend on particle size reduction), but also to the post-duodenal segments, more specifically the caecum. However, based upon the descending part of faecal excretion curves after pulse dosing into the duodenum, they observed residence times in the caecum of 2.7 h for Europium-labelled faecal particles. This corresponds with our faecally determined CMRT2 of Co and Cr (Table 5) and the CMRT of markers determined after pulse dosing into the ileum (Table 4).

Pellikaan *et al.* (2004[b]) reported CMRT values following pulse doses into the ileum between 2.3 and 3.9 h. In contrast, they observed that especially the faecally determined CMRT2 values (upon rumen pulse dose) of ^{13}C-markers were distinctly higher, and appeared to be more comparable with the TMRT determined after ileal pulse doses. In the current experiment the CMRT (ileal, Table 4) of Co (2.8 h) and Cr (3.0 h) were similar to those of the faecally determined CMRT2 (Table 5), but ^{13}CDM showed a 2.5-h difference. By determining ileal excretion patterns the influence of the large intestine compartment could be excluded. The ileally determined K_2 of Co and Cr (Table 5) were extremely high and resulted in very low CMRT2 values. However, this was probably an artefact caused by an insufficient number of data points before PCT was reached. In contrast, the ileal determined CMRT2 of ^{13}C-markers (3.1 to 4.8 h, Table 5) was distinctly higher than the external markers ($0.097 \geq P \geq 0.016$) and differed significantly from zero ($P \leq 0.004$). Comparing the faecally determined

CMRT2 values with those obtained from ileum sampling resulted in a 0.9-h difference for ^{13}CDM and 5.3-h for ^{13}CNDR. This implies that depending on marker type there is a considerable influence of another 'second' slowest mixing compartment, other than the caecum-large intestine compartment, which is confounded in faecally derived CMRT2.

The time delay of the internal markers observed here agrees with earlier results (Pellikaan *et al.*, 2004[b]) and is probably in part associated with rumen-related processes like time required for particle size reduction as suggested by Mambrini and Peyraud (1997) and Wylie *et al.* (2000). The ^{13}C-labelled grass in the current study was cut to a size of 3 to 5 cm before entering the rumen whilst Cr-NDF was ground to pass a 0.5-mm sieve, which explains an important part of the differences between these markers. Besides the internal marker particles are subject to digestion and their functional specific weight may have retarded their passage out of the rumen. Mambrini and Peyraud (1994) observed that the model proposed by Dhanoa *et al.* (1985) generated higher TT and lower CMRT2-values compared to their own model calculations, as described in the previous section. Re-calculation of the TT based on the average time between the moment of marker detection in a sample and the moment at which the previous sample was collected, gave transit times of 11.9, 6.0 and 9.6 h (Co, Cr, ^{13}C), showing no evidence of overestimation of TT by the model. In general the TT is regarded to be the non-mixing displacement flow between dose and sampling site (Pond *et al.*, 1988; Wylie *et al.*, 2000), hence, resembling the residence time related to the tubular sections of the intestinal tract. Therefore, TT values (Table 5) can be used to compare with the small intestinal residence times ($CMRT_{SI}$) calculated from the difference in TMRT after ileal and abomasal pulse dosing (Table 4). The $CMRT_{SI}$ for Co (5.3 h), Cr (6.9 h) and ^{13}CDM (7.1 contrasted considerably with their respective faecally determined TT (8.8, 11.9 and 12.7 h). Therefore, the differences between faecal TT and $CMRT_{SI}$ (respectively 3.5, 5.0 and 5.6 h) are likely associated with the large intestine compartment. The ileal determined TT-values (Table 5) should theoretically represent the post-rumen tubular sections, excluding the large intestinal section and therefore, should be similar to the $CMRT_{SI}$. With respect to Cr and ^{13}CDM the ileal TT tended to higher values than the $CMRT_{SI}$, which agrees with earlier data reported by Pellikaan *et al.* (2004[b]). This indicates that TT cannot be solely assigned to the delay in digesta flow caused by the intestines but that, depending on experimental treatments and type of markers used, a variable part can be related to the non-mixing delay within the mixing compartments. The difference in faecal and ileal determined TT and PCT between liquid phase and particle phase markers suggests that liquid and particle phase show a different behaviour in the post-ruminal sections of the intestinal tract. This corresponds with earlier observations (O'Connor *et al.*, 1984) and matches with our own observations after abomasal pulse dosing.

In Situ Degradation Characteristics

The inclusion of a lag time (*T*) in the model that describes degradation in the nylon bag resulted in general in improved coefficients of determination, which agrees with the

observation of Robinson *et al.* (1986) who studied degradation characteristics of perennial ryegrass hay. Compared to their work, the *T*-value of NDF (7 min, ± 16 min; Table 6) in the current study was lower and not significantly deviating from zero. *T*-values of the other fractions did differ from zero with the largest effect for the protein fraction (CP) (T = 1.8 h). This indicates that the fermentative degradation of CP is more delayed than the other fractions, but is followed by a period with a significantly higher rate of fermentation (K_d = 0.049/h). Part of this delayed lag time may be associated with the rate at which feed particles are being colonized by the microbes after introduction into the rumen. However, it would then be plausible to expect that more pronounced lag times would also occur in the other fractions, especially in the NDF-fraction. Our observed non-washable potentially degradable fractions (D) agree with literature (Robinson *et al.*, 1986; Valk *et al.*, 1996; Tóthi, 2003), but estimates for K_d tend to be rather low. Tóthi (2003) observed considerable higher fractional degradation rates in the DM (0.063/h), OM (0.056/h), CP (0.086/h) and NDF (0.046/h). Their grass was allowed to regrow for 8 to 13 days in contrast to the 44-day regrowth period in the current experiment, which resulted in considerably lower NDF (mean 375.1 g/kg DM) and a higher CP (mean 211.2 g/kg DM) contents. Also the level of fertilizer application is known to affect the degradation characteristics of grass (Valk *et al.*, 1996). With fertilizer regimes set between 150 to 450 kg N/ha and regrowth periods of 18 to 35 days, they reported higher fractional degradation rates compared to our observations. In the current experiment 110 kg N/ha per year was applied to the pasture. This moderate level of N-application combined with an extended period of regrowth attributed to the lower K_d-values.

Feed Intake in Relation to Passage and Degradation Characteristics

The use of feeding balances allows studying the feed intake behaviour of animals more detailed and gives insight in the 'non-steady state' filling pattern of the rumen compartment. From the decline in roughage weight per time interval an absolute intake rate (IR; in kg DM/h) and a fractional intake rate constant (IRf; /h) were calculated, the latter scaled to the daily DMI. In an experiment of Bosch *et al.* (1992) early and late lactation cows received diets of silages varying in NDF content combined with high (± 40%) and low (± 8%) proportions of concentrates. Calculation of the IR and IRf from their observations (min/kg DM) shows values ranging from 1.6 to 2.8 kg DM/h (IR) and 0.10 to 0.20/h (IRf), which agrees with our observations. However, according to Bosch *et al.* (1992) no silage effect was found with regard to rumination and eating time (min/kg DM) and both items only differed between concentrate levels when corrected for the amount of dietary concentrate. Robinson *et al.* (1987) proposed a model where the rate of intake was expressed relative to rumen pool size, assuming linearity in DMI through the day. However, in the current study no quantitative measurements on rumen pool sizes were taken and for calculating the IR and IRf linearity was only assumed within separate eating events. This makes comparison and interpretation of this data with literature difficult (e.g. Bosch, 1991; Rinne *et al.*, 1997; Kovács *et al.*, 1998). Chilibroste *et al.* (1997) allotted cows to increasing time intervals during

which the animals could graze, recorded the effective grazing time and determined the herbage intake based on sward measurements and rumen evacuations. The IR based on sward measurements before and after grazing resulted in higher IR-values (3.5 ± 1.0 kg DM/h; mean ± SD) compared to our observations, however, the results obtained from rumen emptying showed comparable IR-values (2.7 ± 0.6 kg DM/h; mean ± SD) to animal 65 with the highest IR. The difference in feed intake pattern clearly shows that animal 65 was more restricted than the other animal, resulting in higher IR and IRf-values, a lower number of eating events and considerably less time spent on eating. Concomitantly, this animal showed distinct periods of feed restriction (Figure 1) following the morning (4.7 h), afternoon (4.9 h) and evening (9.5 h) feeding. Cows assigned to different intervals of starvation (16.5 vs. 2.5 h) were reported to significantly increase the level of DMI by about 2 kg DM when allowed to voluntary grazing (Chilibroste *et al.*, 1997). The animals also prolonged their time spent on grazing and, hence, showed that starvation time had no distinct effect on the intake rate with respective IR-values for long and short starvation of 2.9 and 2.6 kg DM/h. In their experiment the time of voluntary grazing following a starvation treatment started at 0900 h, hence, short-term starved animals were excluded from the pasture at 0630 h. It could well be that an undefined 'natural' starvation time overnight was confounded with the short 'experimental' starvation treatment, thus obscuring a possible effect on feed intake rate. On the other hand, it might be that animals subjected to one-time occurring starvation periods in combination with a history of *ad libitum* access to feed as described by Chilibroste *et al.* (1997), have no long-term physiological incentive to adapt their DM intake rate. In the current experiment, the daily offered DM was equal for individual animals and based on the animal with the lowest intake. Therefore, animal 65 had to adapt to a more 'chronic' type of feed restriction during the 3-week experimental period time forcing the animal to change its feed intake behaviour.

The difference in feed intake behaviour in response to deviations from *ad libitum* feeding level might affect rumen conditions, and hence, influence passage characteristics. Due to the short time spend on eating and concomitant high rates of intake of animal 65, at each meal this animal theoretically 'pulse doses' itself with unlabelled material. Potentially, this can cause sigmoid-like fluctuations in the excretion pattern of ^{13}C-markers. Coates *et al.* (1990) observed such effects in sheep receiving alternating diets containing primarily C_3 or C_4-plant species at different time intervals (48, 24 and 12 h). An increase in time interval amplified the sigmoidal pattern of faecal δ^{13}C-excretion, and alternating diets at 12-h intervals resulted in an average faecal enrichment level of –20 δ^{13}C vs. PDB with small but distinct diurnal deviations from this point. With regard to our data, an increase in the mean prediction error and/ or a shift its decomposed fractions (Bias, Line, Rand), could be indicative for such effects. However, the data in our experiment is based on only 2 animals and not truly adequate for testing. MPE-values calculated from faecally and ileally determined curve fits did not differ between animals ($P \geq 0.172$). The over-all fractional rumen passage rates (K_1) of animals 61 and 65 determined from ileal (0.043 vs. 0.050/h, $P = 0.303$) and faecal (0.042 vs. 0.062/h, $P = 0.204$) excretion patterns did not show distinct differences, which is also true for the other

estimated parameters (A, K_2, N; Table 5). Although differences in feed intake patterns were considerable between animals, this had no significant influence on marker excretion patterns under the current experimental conditions.

Clearance Rate of Rumen Contents

Within the Dutch protein evaluation system (DVE/OEB-system) the bypass part of the feed protein is determined by feed specific fractional degradation rates and fixed values for fractional passage rates, 0.045/h for roughages and 0.060/h for concentrates (Tamminga *et al.*, 1991). Hence, within feed type the variation in the effective escape is only influenced by the fractional rate constant of degradation. However, it is increasingly realized that passage rates do vary and depend on roughage type and conservation method (Huhtanen and Hristov, 2001; Lund, 2002), roughage quality (Bruining and Bosch, 1991; Rinne *et al.*, 1997; Tjardes *et al.*, 2002), diet composition and the level of feed intake (Tamminga *et al.*, 1989; Huhtanen and Kukkonen, 1995; Pellikaan *et al.*, 2004[b]). The clearance of the rumen material is a function of passage and degradation, and hence, the rate of clearance (K_c) equals the sum of the fractional rate constants of passage and digestion. A combination of the K_d-values obtained in the current study (Table 6) with the fixed rate constant for passage of 0.045/h results in average ruminal clearance rates (K_c) for DM, NDF and CP of respectively 0.071, 0.068 and 0.094/h. However, by introducing feed specific fractional passage rates obtained from respectively ^{13}CDM, ^{13}CNDR and ^{13}CNDS, the respective values of K_c for the DM, NDF and CP become 0.051, 0.034 and 0.084/h. The K_c-value of DM is in line with observations in grass silage fed cows (Bosch, 1991) and cows grazing more matured grass (Chilibroste, 1999). In experiments where cows received different types of roughages, Lund (2002) observed ruminal NDF clearance rates in a range of 0.025 to 0.044/h. However, comparing data with observations of Tóthi (2003) where animals under grazing conditions received different sources of starch, our values for ruminal NDF clearance are distinctly lower whilst ruminal CP clearance is considerable higher. Tóthi (2003) explained the low clearance rate of CP due to an increased level of N being captured by the rumen microbes caused by the amount of starch in the diets (3 kg pure starch/d).

Our calculations suggest that fractional passage rates as estimated from the external marker Cr-NDF, give an overestimation of the fractional passage rates of the DM and NDF fractions.

Conclusions

The faecal excretion patterns of internal markers (^{13}CDM, ^{13}CNDR, ^{13}CNDS) clearly differed from those of the external markers after pulse dosing in the rumen. Between the internal markers, ^{13}CNDR gave significant longer rumen and total tact residence times compared to the others. This indicates that the use of ^{13}C as an internal marker enables to discriminate for feed component specific passage kinetics. Only after abomasal pulse dose,

particle phase markers tended to differentiate from the liquid phase in the post rumen section of the intestinal tract. Based on a comparison of passage and degradation results with clearance rates observed in other studies, the conventionally used external particle phase marker (Cr-NDF) overestimates fractional passage rate constants for both the DM and cell wall fractions. Feed intake patterns between animals showed considerable differences in intake rate (IR), the effective time spend on eating and number of eating events. However, this did not seem to affect excretion patterns measurably. A comparison of the mean prediction errors (MPE) showed no differences in accuracy between curve fit results of external vs. internal markers, indicating that the use of ^{13}C as an internal marker in passage studies is a viable tool.

Acknowledgements

This work was co-financed by the Dutch Commodity Board of Feedstuffs, which is gratefully acknowledged. The authors kindly acknowledge the technical assistance of Mr. W. van Gestel, Mr. A. van den Dool, Mr. L. Bijl, and the analytical assistance of Miss. J. M. Muylaert, Mrs. M. van‘t End and Miss. G. Post.

Literature Cited

AFRC. 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. AFRC technical committee on responses to nutrients. Report no. 11. Nutr. Abstr. Rev. (Ser. B). 68, 937-989.

Baldwin, R.L. 1995. Modeling ruminant digestion and metabolism. Chapman & Hall Ltd., London, UK.

Bibby, J., and H. Toutenberg. 1977. Prediction and improved estimation in linear models. London: John Wiley and Sons.

Bosch, M.W. 1991. Influence of stage of maturity of grass silages on digestion processes in dairy cows. Ph.D. Diss., Wageningen Agricultural University, The Netherlands.

Bosch, M.W., S.C.W. Lammers-Wienhoven, G.A. Bangma, H. Boer, and P.W.M. van Adrichem. 1992. Influence of stage of maturity of grass silages on digestion processes in dairy cows. 2. Rumen contents, passage rates, distribution of rumen and faecal particle and mastication activity. Livest. Prod. Sci. 32: 265-281.

Boudon, A., and J.L. Peyraud. 2001. The release of intracellular constituents from fresh ryegrass (*Lolium perenne* L.) during ingestive mastication in dairy cows: effect of intracellular constituent, season and stage of maturity. Anim. Feed Sci. Techn., 93, 229-245.

Bruining, M., and M.W. Bosch. 1991. Ruminal passage rate as affected by Cr-NDF particle size. Anim. Feed Sci. Technol. 37:193-200.

Chilibroste, P. 1999. Grazing time: the missing link. A study of the plant-animal interface by integration of experimental and modeling approaches. Ph.D. Diss., Wageningen Agricultural University, The Netherlands.

Chilibroste, P., S. Tamminga, H. Boer. 1997. Effects of length of grazing session, rumen fill and starvation time before grazing on dry-matter intake, ingestive behaviour and dry-matter rumen pool sizes of grazing lactating dairy cows. Grass Forage Sci., 52: 249-257

Coates, D. B., A. P. A. Van der Weide, and J.D. Kerr, J.D. 1991. Changes in faecal delta^{13}C in response to changing proportions of legume (C_3) and grass (C_4) in the diet of sheep and cattle. J. Agric. Sci. 116 (2):287-295.

Colucci, P.E., G.K, MacLeod, W.L. Grovum, I. McMillan, and D.J. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 73, 2143-2156.

Dhanoa, M.S., R.C. Siddons, J. France, and D.L. Gale. 1985. A multicompartmental model to describe marker excretion patterns in ruminant faeces. Br. J. Nutr. 53:663-671.

Dijkstra, J., and J. France. 1996. A comparative evaluation of models of whole rumen function. Ann. Zootechnie., 45:175-192.

Dijkstra, J., J. France, and D.R. Davies. 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. J. Dairy Sci. 81, 3370-3384.

Gasa, J., K. Holtenius, J.D. Sutton, M.S. Dhanoa, and D.J. Napper. 1991. Rumen fill and digesta kinetics in lactating Friesian cows given two levels of concentrates with two types of grass silage ad lib. Br. J. Nutr. 66, 381-398.

Goelema, J.O., M.A.M. Spreeuwenberg, G. Hof, A.F.B. van der Poel, and S. Tamminga. 1998. Effect of pressure toasting on the rumen degradability and intestinal degradability of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Anim. Feed Sci. Technol. 76:35-50.

Huhtanen, P., and A.N. Hristov. 2001. Estimating passage kinetics using fibre-bound ^{15}N as an internal marker. Anim. Feed Sci. Technol. 94:29-41.

Huhtanen, P., and U. Kukkonen. 1995. Comparison of methods, markers, sampling sites and models for estimating digesta passage kinetics in cattle fed at two levels of intake. Anim. Feed Sci. Technol. 52:141-158.

Kokkonen, T., M. Tuori, and L. Syrjälä-Qvist. 2000. Effect of silage dry matter content and rapeseed meal supplementation on dairy cows 2. Rumen fermentation and digesta passage rate. Anim. Feed Sci. Technol. 84:229-242.

Kovács, P.L., K.-H. Südekum, and M. Stangassinger. 1998. Effects of intake of a mixed diet and time postfeeding on amount and fibre composition of ruminal and faecal particles and on digesta passage from the reticulo-rumen of steers. Anim. Feed Sci. Technol. 71:325-340.

Lund, P. 2002. The effect of forage type on passage kinetics and digestibility of fibre in dairy cows. Ph.D. Diss., The Royal Veterinary and Agricultural University Copenhagen, Denmark.

Mambrini, M., and J.L. Peyraud. 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet. Reprod. Nutr. Dev. 34:9-23.

Mambrini, M., and J.L. Peyraud. 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculations based on faecal collection. Reprod. Nutr. Dev. 37:427-442.

O'Connor, J.D., P.H. Robinson, C.J. Sniffen, and M.S. Allen. 1984. A gastro-intestinal tract simulation model of digesta flow in ruminants. Pages 102-121 in Techniques in particle size analysis of feed and digesta in ruminants. Ed. P.M. Kennedy. Can. Soc. Anim. Sci. Occ. Publ. 1, Edmonton, Alberta, Canada.

Owens, F.N., and A.L. Goetsch. 1986. Digesta passage and microbial protein synthesis. In: Control of Digestion and Metabolism in Ruminants, pp. 196-223 [LP Milligan, WL Grovum and A Dobson, editors] Prentice-Hall, Englewood Cliffs.

Pellikaan, W.F., H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven, and S. Tamminga. 2004[a]. ^{13}C-enrichment of cell wall fractions of rye grass (*Lolium perenne* L.) and implications for its use as digesta marker. Submitted to J. Anim. Sci.

Pellikaan, W.F., J. Dijkstra, S. Tamminga, H. Boer, S.C.W. Lammers-Wienhoven, W.J.H. Van Gestel, and G. Hof. 2004[b]. Passage of ^{13}C-labelled grass silage through the gastro-intestinal tract of dairy cows at two levels of feed intake. Submitted to J. Anim. Sci.

Pond, K.R., W.C. Ellis, J.H. Matis, H.M. Ferreiro, and J.D. Sutton. 1988. Compartment models for estimating attributes of digesta flow in cattle. Brit. J. Nutr. 60:571-595.

Poore, M.H., J.A. Moore, T.P. Eck, and R.S. Swingle. 1991. Influence of passage model, sampling site, and marker dosing time on passage of rare earth-labeled grain through Holstein cows. J. Anim. Sci. 69:2646-2654.

Rinne, M., P. Huhtanen, and S. Jaakkola. 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol. 67:19-35.

Robinson, P.H., J.G. Fadel, and S. Tamminga. 1986. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Anim. Feed Sci. Technol. 15:249-271.

Robinson, P.H., S. Tamminga, and A.M. Van Vuuren. 1987 Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. Livest. Prod. Sci. 17, 37-62.

Südekum, K.H., W. Ziggers, N. Roos, H. Sick, S. Tamminga, and M. Stangassinger. 1995. Estimating the passage of digesta in steers and wethers using the ratio of ^{13}C to ^{12}C and titanium(IV)-oxide. Isotopes Environ. Health Stud. 31:219-227.

Svejcar T.J, M.B. Judkins, and T.W. Boutton. 1993. Technical note: labeling of forages with ^{13}C for nutrition and metabolism studies. J. Anim. Sci. 71:1320-1325.

Tamminga, S., P.H. Robinson, M. Voigt, and H. Boer. 1989. Rumen ingesta kinetics of cell wall components in dairy cows. Anim. Feed Sci. Technol. 25:89-98.

Tamminga, S., W.M. Van Straalen, A.P.J. Subnel, R.G.M. Meijer, A. Steg, C.J.G. Wever, and M.C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40:139-155

Tjardes, K.E., D.D. Buskirk, M.S. Allen, N.K. Ames, L.D. Bourquin, and S.R. Rust. 2002. Neutral detergent fiber concentration of corn silage and rumen inert bulk influences dry matter intake and ruminal digesta kinetics of growing steers. J. Anim. Sci. 80:833-840.

Tóthi, R. 2003. Processed grains as a supplement to lactating dairy cows. Ph.D. Diss., Wageningen University, The Netherlands.

Udén, P., P.E. Colucci, and P.J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31:625-632.

Valk, H., I.E. Kappers, and S. Tamminga. 1996. *In sacco* degradation characteristics of organic matter, neutral detergent fibre and crude protein of fresh grass fertilized with different amounts of nitrogen. Anim. Feed Sci. Technol. 63:63-87.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van Vuuren, A.M. 1993. Digestion and nitrogen metabolism of grass fed dairy cows. PhD Thesis, Wageningen Agricultural University, The Netherlands.

Wylie, M.J., W.C. Ellis, J.H. Matis, E.M. Bailey, W.D. James, and D.E. Beever. 2000. The flow of forage particles and solutes through segments of the digestive tracts of cattle. Brit. J. Nutr. 83:295-306.

Yang, W.Z. K.A. Beauchemin, and L.M. Rode. 2001. Barley processing, forage:concentrate, and forage length effects on chewing and digesta passage in lactating cows. J. Dairy Sci. 84:2709-2720.

Chapter 5

Passage of ^{13}C-Labelled Grass Silage Differing in Quality Through the Gastro-Intestinal Tract of Dairy Cows

W.F. Pellikaan, S. Tamminga, G. Hof[1] H. Boer, M. Roordink, and J. Dijkstra

Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands

Submitted to Journal of Dairy Science

[1] in memory of G. Hof

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W.F. Pellikaan, S. Tamminga, G. Hof, H. Boer, M. Roordink, and J. Dijkstra

Abstract

The stable isotope of carbon (^{13}C) was used to obtain feed specific fractional passage rates of two grass silages differing in digestibility in dairy cows. Three rumen and ileum cannulated early lactation HF cows were used to evaluate the passage behavior of the ^{13}C-labelled silages compared to that of Cr-NDF and Co-EDTA. Animals were alternately assigned to diets of low (GSL) or high (GSH) digestible grass silage and concentrates in a fixed ratio (54:46). Successively, animals received pulse doses of ^{13}C-labelled grass silage, Cr-NDF and Co-EDTA into the ileum, the abomasum and the rumen. Faecal excretion patterns of the DM (^{13}CDM), Cr and Co were determined after ileal and abomasal pulse doses. After rumen pulse doses, faecal and ileal excretion curves were established of the DM, the cell wall (^{13}CNDR) and the non-cell wall fraction (^{13}CNDS), and of Cr and Co. Faecally determined passage rates after an ileal pulse dose showed no effect of markers and silage type. After abomasal pulse dosing, differences in passage rates between markers became more pronounced but between silages no differences were observed. Feacally and ileally determined passage rates from the rumen of ^{13}CDM and ^{13}CNDR were considerably lower than those of Cr and Co. Fractional passage rates obtained with ^{13}C tended to be higher for GSH than GSL. Combined information on the moment when marker excretion reaches its peak, the transit times, compartmental residence times and total mean residence times showed more pronounced differences between silages for the internal markers than the external markers. ^{13}C was a more sensitive marker to distinguish differences in passage behavior between the feeds and their components, and in relation to ruminal degradation and clearance more rational passage rates were generated.

Keywords: ^{13}C-isotope, Passage rate, Grass silage quality, Dairy cow

Introduction

Modern protein evaluation systems for ruminants describe the supply of protein to the animal as the sum of a microbial component, a bypass feed protein component, a correction for the endogenous losses, and the digestibility of these components in the small intestine (Vérité *et al.*, 1987; AFRC 1992; Tamminga *et al.*, 1994). The rumen escape part of feed protein is determined by feed specific fractional degradation rates and fixed fractional passage rates. However, many studies indicate that fractional passage rates do vary and are affected by roughage type, method of conservation and diet composition (Huhtanen and Hristov, 2001; Yang *et al.*, 2001; Lund, 2002), roughage quality (Bosch *et al.*, 1992; Rinne *et al.*, 1997; Kokkonen *et al.*, 2000), and level of feed intake (Tamminga *et al.*, 1989; Colucci *et al.*, 1990; Huhthanen and Kukkonen, 1995). However, most studies have been carried out with external markers and therefore, do not give information of treatment effects on feed or feed component specific fractional passage rates. Knowledge about feed or feed component specific retention times is essential for the future development of so-called “nutrient based” feed evaluation

systems (Baldwin, 1995; AFRC, 1998; Dijkstra *et al.*, 1998), where fractional passage rates form important factors as they determine the site of degradation and the efficiency of microbial protein synthesis (Dijkstra and France, 1996). To acquire information on feed specific passage rates internal markers are preferred to external markers as they are representative for the *in vivo* situation. Huhtanen and Hristov (2001) and Pellikaan *et al.* (2004[b,c]) used respectively ^{15}N and ^{13}C as internal markers to quantify passage kinetics originating from different roughage sources and feed fractions. Both studies showed that the use of stable isotopes as internal markers provide advantages to study passage kinetics compared to traditionally used external markers.

The main objective of the present study was to assess feed specific fractional passage rates of feed particles originating from grass silages of different digestibility through different compartments of the gastro-intestinal tract of dairy cows. In addition, an attempt was made to evaluate relations between passage rates and degradation rates. The traditionally used external markers Co-EDTA and Cr-NDF were included to compare and evaluate the behavior of the internal marker.

Materials and Methods

Pasture Selection and Marker Preparation

Two uniform pastures were selected at the experimental farm 'De Ossekampen' of Wageningen University. The pastures, sown 2 year before with a mixture of diploid and triploid rye grass (*Lolium perenne* L.) and timothy grass (*Phleum pratense* L.) were divided into 2 sections and assigned to 2 levels of N fertilization; 100 vs. 50 kg N/ha. The sections were assigned to respectively a 6-week and a 12-week regrowth period to the moment of ensiling. Thus, a silage of high digestibility and high CP/NDF ratio (GSH) and a silage of low digestibility and low CP/NDF ratio (GSL) were created (Table 1). Within each section two small homogenous plots (2.15 m^2/ plot) were selected and successively labelled with $^{13}CO_2$ at 6 (GSH) and 8 (GSL) different days during the period of regrowth. Details on the labelling procedure are described elsewhere (Pellikaan *et al.*, 2004[a]). The day following a final labelling treatment, the plots and pasture were harvested, and ensiled, subjecting the ^{13}C-labelled grass to the same ensiling conditions than the silage. Two weeks prior to the start of the animal trial the ^{13}C-labelled ensiled materials were recovered from the silages, cut manually to an approximate size of 3 cm using a paper cutter, thoroughly homogenised and stored in airtight plastic bags at –20°C. Sub samples were analysed for DM, NDF and neutral detergent residue (NDR), for total carbon content (TC) and ^{13}C:^{12}C-ratio in the DM (^{13}CDM) and NDR ($^{13}CNDR$). The non-cell wall fraction (NDS) and its enrichment ($^{13}CNDS$) were derived from the difference between DM and NDR. Details on sample treatment, chemical analyses and calculations are reported by Pellikaan *et al.* (2004[a,b]). In addition to the ^{13}C-labelled marker Co-EDTA and Cr-NDF were used. Both markers were prepared as described

Table 1. Ingredients and chemical composition of the compound feed and the grass silage of lower (GSL and higher (GSH) digestibility.

Item	Compound Feed[1]	GSL[2]	GSH[2]
Ingredients			
Wheat	204.1		
Palm kernel expeller, crude fiber < 220	150.0		
Sunflower seed, extracted, crude fiber 160 – 200	26.2		
Soy hulls, crude fiber > 310	1.5		
Coconut expeller, crude fat < 100	150.0		
Beet pulp, sugars 100 – 150	400.0		
Vinasse, crude protein < 250	50.0		
Phosphoric acid limestone,	7.6		
Salt	3.0		
Minerals (Mervit rundvee 31)[4]	7.5		
Chemical composition			
DM, g/kg	900.0	450.0	341.0
OM	917.0	880.0	890.0
CP	148.0	124.0	182.0
DVE[5]	109.0	56.0	74.0
OEB[5]	-17.0	1.0	54.0
OMd, %[5]	-	71.7	79.8
NDF[3]	197.0	511.6	408.9
ADF[3]	101.0	300.9	258.4
ADL[3]	28.0	30.2	20.6
Starch	139.0	-	-
Sugars	79.0	91.0	79.0
NEL, MJ/kg DM	7.2	5.5	6.4

[1] Ingredients and chemical composition determined through linear programming, units in g/kg of DM unless specified otherwise.
[2] DM, OM, CP, DVE, OEB, sugars and NEL determined through near infrared reflection spectroscopy (NIRS), Blgg, Oosterbeek, The Netherlands. Units in g/kg of DM unless specified otherwise.
[3] NDF analysed according to a modified method of Van Soest *et al.* (1991) as described by Goelema *et al.* (1998), ADF and ADL analysed as described by Van Soest (1973).
[4] Mineral premix on basis of limestone, PRE-MERVO, Utrecht, The Netherlands.
[5] DVE = true protein digested in the small intestine, OEB = degraded protein balance, OMd = organic matter digestibility coefficient; units in the Dutch protein evaluation system as defined by Tamminga *et al.* (1994).

by Udén *et al.* (1980). With regard to Cr-NDF wheat straw was used as fiber source and after drying Cr-NDF was ground to pass a 0.5-mm screen.

Passage Experiment

Animals and housing. Three third lactation Holstein-Friesian dairy cows fitted with a rumen fistula (Type 1C, Bar Diamond, Inc., Parma, Idaho, USA) and a T-shaped ileum cannula were used. The ileal cannulas were constructed from silicone tubing (i.d./o.d. 25/34 mm) and placed at 20 to 30 cm proximal to the ileo-caecal valve. The experiment was carried out at the experimental farm "De Ossekampen" of Wageningen University where animals were housed in a tie-stall. At the start of the experiment animals were 99 ± 22 DIM (mean ±

SD), had a BW of 578 ± 7 kg (mean ± SEM) and produced 25.3 ± 1.2 kg (mean ± SEM) fat and protein corrected milk/d. Handling of animals and experimental layout were submitted to and approved of by an ethical committee, and were executed in accordance with Dutch legislation on the use of experimental animals.

Experimental layout and dietary treatment. The experiment consisted of 4 periods in which the 3 animals were randomly assigned to one of the 2 treatments (GSH or GSL). An experimental period comprised a 3-week adaptation period followed by 1 measurement week. At the end of a measurement week animals were directly re-allocated to new diets to start the subsequent adaptation period. Thus, animals received each dietary treatment twice. Prior to an experimental period an adequate amount of both silages was homogenised in a large roughage mixer, weighed in the required daily portions into plastic bags and stored at –20°C to ensure constant diet quality throughout a period. Two days prior to feeding bags were removed from the freezer to defrost. In addition animals received a specially fabricated compound feed (Table 1) in a preset fixed proportion of the total daily DMI. The ingredients of the compound feed were carefully selected to keep the level of naturally occurring ^{13}C-enrichment similar to that of the silage in the diet. The total daily DMI at the start of the experiment was 17.7 ± 0.3 kg/d (mean ± SEM) with 45.8 ± 0.9% (mean ± SEM) as concentrates. Overall averages are summarised per silage type in Table 2. The roughage to concentrate ratio was maintained during the total experimental period by minimizing the feed residues, but changes in daily amounts of feed offered were kept to a maximum of 5%. The daily amounts of roughage and compound feed were offered in five equal portions. Roughage was offered at 0600, 1000, 1400, 1800 and 2200 h., concentrates were given 30 min later. Orts were collected daily at 0600 h and weighed. Animals had free access to water and a commercial available mineral lick (KNZ®Rundvee, AKZO NOBEL SALT).

Pulse dose treatments. Depending on their dietary treatment (GSH vs. GSL), animals received pulse doses of a corresponding ^{13}C-labelled silage in combination with Cr-NDF and Co-EDTA in consecutively, the ileum, the abomasum and the rumen. Prior to pulse dosing into the ileum and abomasum, the frozen and cut labelled grass silage was further cut to an approximate size of 0.5 mm and allowed to acclimatise to ambient temperature for 24 h in

Table 2. Animal and diet characteristics (mean ± SEM) for different treatment groups (GSL, GSH), where $P < 0.05$ indicates significant difference between treatments.

Item	GSL	GSH	SEM	$P < \|T\|$
Body weight, kg	565.4	559.3	5.0	0.437
Milk production, kg	17.7	20.6	0.3	0.002
Fat, %	5.43	4.84	0.21	0.113
Protein, %	3.06	3.09	0.06	0.790
Total DMI, kg	15.5	16.2	0.1	0.003
Concentrate ratio, % of DMI	47.2	45.1	0.3	0.002
DMd[1], %	70.8	75.6	0.5	0.002

[1] DMd = dry matter digestibility coefficient total diet.

sealed plastic bags. Twelve h prior to introduction into the intestinal tract, the exact amounts of ^{13}C-labelled grass, Cr-NDF and Co-EDTA were soaked overnight in a 0.4% carboxy methylcellulose solution (CMC, Fluka Chemie GmbH, Switzerland); for the ileum and abomasum in respectively a 2.0 and 2.5 L CMC-solution. The marker containing solution was quantitatively introduced into the ileum and abomasum using a 1.5-m long silicon tube (i.d. 25 mm). To place the tube into the abomasum part of the rumen content was evacuated. Subsequently, the tube was manually fixed into the omasal-abomasal orifice. Before introduction of the ^{13}C-labelled grass into the rumen, the material was defrosted overnight in a refrigerator and subsequently acclimatised to ambient temperature in sealed plastic buckets. The total rumen content was evacuated, mixed thoroughly with the three markers and placed back quantitatively into the rumen cavity. Table 3 summarises the composition of pulse doses for individual animals.

Sampling and chemical analyses. The compound feed was sampled each time portions for individual animals were weighed. Orts were sampled daily, dried at 60°C and pooled by animal per period. The grass silages were sampled per period. Per silage, part of these samples were pooled and analysed by use of a standard NIRS procedure (Table 1). The remaining part of the silage samples were pooled and dried at 60°C. These materials were then ground to pass a 1-mm screen (Wiley mill, T. Peppink & Zn., Machinefabriek, Amsterdam) and analysed for DM (ISO 6496), ash (ISO 5984), NDF, ADF and ADL. The NDF was analysed according to a modified method of Van Soest *et al.* (1991) as described by Goelema *et al.* (1998). The ADF and ADL were analysed as described by Van Soest (1973).

Table 3. Composition of pulse doses for different treatment groups (GSL, GSH) and sites (ileum, abomasum, rumen) of the gastro-intestinal tract[1,2].

			GSL[3]			GSH[3]		
Item	Co	Cr	^{13}CDM	^{13}CNDR	^{13}CNDS	^{13}CDM	^{13}CNDR	^{13}CNDS
Ileum	268.7	241.3	105.9			99.6		
	(0.8)	(0.8)	(0.7)			(0.3)		
Abomasum	456.4	401.8	145.3			176.6		
	(2.5)	(1.1)	(0.9)			(0.4)		
Rumen	4455.6	3978.3	677.3	320.4	357.0	704.5	292.7	411.8
	(2.2)	(2.0)	(1.0)	(0.5)	(0.5)	(0.3)	(0.1)	(0.2)
			---------- Isotopic enrichments[4] ----------					
Labelled grass silage, %			1.654	1.567	1.765	1.968	1.883	2.040
Unlabelled grass silage, %			1.078	1.079	1.077	1.079	1.079	1.079

[1] Co-EDTA contained 148.5 g Co/kg ; Cr-NDF contained 39.8 g Cr/kg.
[2] Amounts given in mg (mean ± SEM) and specified by treatment group; GSL = grass silage of low digestibility, GSH = grass silage of high digestibility.
[3] Pulse dosed quantities of ^{13}C in mg for different fractions (DM, NDR and NDS) corrected for the naturally occurring amounts of ^{13}C.
[4] Isotopic enrichment of different fractions (DM, NDR, NDS) expressed in atom percentages ^{13}C (At%^{13}C) for labelled grass and grass under natural conditions.

In addition, representative roughage and compound feed samples were taken to be evaluated at a later stage for *in situ* degradation characteristics. The roughage samples were stored at –20°C and the compound feed at 4°C. Milk production and quality were monitored on a daily basis throughout the total experimental period and samples were analysed for fat and protein using NIRS (Milk Control Station, Zutphen, The Netherlands).

Faecal samples were taken at each defecation starting directly after administration of the markers into the ileum and continued till approximately 100 h post ruminal pulse dose administration. Abomasal pulse dose was given 24 h after the ileal pulse dose and ruminal pulse dose followed at 30 h post abomasal pulse dose. From each defecation, the faeces was quantitatively collected, weighed, homogenised and representatively sampled. Ileum chyme samples were only collected after the administration of markers into the rumen, using 1-L plastic bottles, connected to the cannula and secured to the animal with a belt. Chyme collection started 3 h after pulse dosing at 3-h intervals during the first 24 h. Thereafter, chyme was collected at 6-h intervals, resulting in a total of 23 samples. The collected chyme was weighed, homogenised and sampled. Faecal and chyme samples were stored at –20°C, and subsequently freeze-dried (FTS, Dura-Dry programmable tray freeze drier), ground to pass a 1-mm screen (Wiley mill) and analysed for DM and NDR, TC in the DM and NDR, and ^{13}CDM and ^{13}CNDR. The NDS and ^{13}CNDS were obtained as described by Pellikaan *et al.* (2004[a,b]).

The NDR-analyses was similar to the method of Van Soest (1991), but filter glass crucibles (Fiber Tec system) were replaced by filter bags (Type F57, porosity 25 μm, Ankom Technology, 2003). Filter bags were filled with 0.50 g of sample (± 0.05 g), heat-sealed for 4 s and pooled to batches of 30 bags. Each batch was washed twice in 900 ml acetone, dried over night and then immersed in preheated commercially purchased ND-reagent (100 ml/bag, batch no. 2002093818, Boom BV), where the bags were thoroughly agitated through the reagent. When nearing 98°C, heat stable α-amylase (1.0 ml/bag; Thermamyl, no. 01319, Labshop VOF) was added and from the moment 100°C was reached the bags were boiled for a 60-min period. To correct for evaporative losses at 20 and 40 min 300 ml of boiling demineralized water was added. After this step the bags were 4 to 5 times thoroughly washed in boiling demineralized water (about 1 L/rinse). Successively, the bags were incubated for 15 min in 2 L of a pre-heated Sorensen phosphate buffer solution (40°C, pH 7) to which α-amylase (1.0 ml/bag; Roche Diagnostics GmbH, 1600 iU/ml) and protease (0.25 ml/bag; Alcalase, Novo Nordisk, 2.4 AU/g) was added. Subsequently, the bags were thoroughly rinsed in an excess of demineralized water (100°C) and washed twice in acetone. From the loss of weight and a correction for blanks the NDR was calculated (Ankom Technology, 2003). The external markers were oxidized by wet-destruction at 350°C for 1 h in an HNO_3 (65%, 874380, Fluka Chemie GmbH) and $HClO_4$ (70-72%, 1005192500, Merck KgaA) solution and absorbency of Cr^{6+} and Co^{3+} was measured at respectively 357.8 and 251.0 nm in a nitrous oxide acetylene flow using an atomic absorption spectrophotometer (SpectrAA.300, Varian Inc., Palo Alto, VS).

One day prior to the marker administrations, at each feeding time three consecutive rumen liquid samples were taken; just before roughage feeding (T0), 30 min later just before the concentrate feeding (T30) and at 1 h after concentrate feeding (T90). About 300 ml of rumen liquid was collected proportionally from a cranial, middle and caudal direction, by lowering a flexible plastic tube attached to a 1-L squirt bottle into a 1-m plastic pipe (o.d. 4 cm) with 1.5-mm holes in the lower 27 cm. The liquid was strained over cheesecloth and temperature corrected pH was directly measured using a Testo 252 (Testo GmbH & Co., Germany) in combination with a closed type pH electrode (HI 1230, Hanna Instruments Sub-samples were taken for VFA (10 mL liquid in 0.5 mL Phosphoric acid, 85%) and NH_3-analyses (5 mL liquid in 5 mL 10% Trichloroacetic acid) and stored at –20°C. Excess fluid was returned to the rumen.

Calculations and statistical analyses. Faecal and ileal excretion patterns of the excess ^{13}C in DM, NDR and NDS were established after correcting the atom percentage ^{13}C (At%^{13}C) for natural enrichment. The declining part of the faecal excretion curves of Co, Cr and ^{13}CDM obtained after administration of pulse doses into the ileum and abomasum, were fitted using a single exponential equation as described by Pellikaan *et al.* (2004[b,c]). Faecal and ileal excretion patterns of markers following from a pulse dose into the rumen were fitted to the nonlinear multi-compartmental model of Dhanoa *et al.* (1985) as described earlier in detail in Pellikaan et al (2004[b,c]). Curve fitting was done using the nonlinear least squares regression procedure PROC NLIN (SAS Inst. Inc., Cary, NC). Initial values for the iterative procedure were obtained through a grid search of the full parameter space. The array of initial values used were assessed from the excretion patterns and solved in 12 to 18 steps for each parameter. Recovery of markers (RP) was calculated by analytical integration of the surface under the faecal excretion curve and expressing the sum of these values relative to the amount of administered marker, with a correction for marker losses due to ileal chyme sampling. Predicted faecal and ileal marker concentrations were compared with the observed values using the mean squared prediction error (MSPE) described by Bibby and Toutenburg (1977). The root MSPE was scaled to the observed mean (Mean Prediction Error, MPE) and decomposed into errors due to over-all bias, errors due to deviation of the regression slope from unity and errors due to random variation.

Model estimates of A (initial marker concentration) and K (fractional rate of passage) and the calculated TT (transit time), TMRT (total mean residence time), PCT (peak concentration time), and RP derived from faecal excretion upon ileal and abomasal pulse dosing were tested using the GLM procedure of SAS. Within the model, markers (Co, Cr, ^{13}CDM) were tested using Eq.1.

$$Y_{ijklm} = \mu + C_i + P_j + D_k + M_l + S_m + (C \times D)_{ik} + (C \times M)_{il} + (C \times S)_{im} + (P \times D)_{jk} + (P \times M)_{jl} + (D \times M)_{kl} + (D \times S)_{km} + (M \times S)_{lm} + (D \times M \times S)_{klm} + e_{ijklm} \quad [1]$$

where Y_{ijklm} represents the dependent variable, μ represents the mean, C_i corrects for the animal effect (i = 1 - 3), P_j corrects for period effect (j = 1 - 4), D_k corrects for pulse dose site (k = 1 - 2), M_l corrects for marker type (l = 1 – 3), S_m corrects for silage type (m = 1 – 2), $(C \times D)_{ik}$, $(C \times M)_{il}$, $(C \times S)_{im}$, $(P \times D)_{jk}$, $(P \times M)_{jl}$, $(D \times M)_{kl}$, $(D \times S)_{km}$, $(M \times S)_{lm}$ and $(D \times M \times S)_{klm}$ refer to the interactions, and e_{ijklm} denotes the error term. After ruminal pulse dosing, model estimates of A, K_1 (fractional passage rate from the slowest compartment), K_2 (fractional passage from the second slowest compartment) and N (number of compartments) of Co, Cr, ^{13}CDM, ^{13}CNDR and ^{13}CNDS, and their calculated TT, CMRT1 ($1/K_1$), CMRT2 ($1/K_2$) , TMRT, PCT and RP were tested with an identical model, but substituting D_k by T_k, where T_k corrects for sample collection site (k = 1 - 2).

Feed intake data, digestibility coefficients and milk yield were tested using the GLM procedure of SAS in a model with cow_i (i = 1 - 3), $period_j$ (j = 1 - 4) and $silage_k$ (k = 1 - 2) as main effects. The effects of sampling time after feeding and silage type on pH, VFA and ammonia concentration were tested in a split-plot arrangement using the MIXED procedure of SAS (Eq.2).

$$Y_{ijklm} = \mu + S_i + C_j + P_k + (S \times C \times P)_{ijk} + FT_l \times TAF_m + (FT \times TAF)_{lm} + (S \times TAF)_{im} + e_{ijklm} \quad [2]$$

with Y_{ijklm} representing the dependent variable, μ representing the mean, S_i (silage type; i = 1 - 2), C_j (animal effect; j = 1 - 3) and P_k (period effect; k = 1 - 4) representing the effects related to the main plots, and FT_l (feeding times; l = 1 - 5), TAF_m (moment of sample collection; m = 1 - 3) and their interaction terms $(FT \times TAF)_{lm}$ and $(S \times TAF)_{im}$ representing the effects related to the subplots within the MODEL statement. By including the interaction term $(S \times C \times P)_{ijk}$ in the RANDOM statement, main plot variables were tested against this three-way interaction term and sub plot variables with their interaction terms were tested against the pooled residual error (e_{ijklm}). Covariance parameters were estimated using the residual maximum likelihood (REML) method and denominator degrees of freedom (DDFM) were estimated using the Satterthwaite approximation to produce accurate F-statistics (SAS Inst. Inc., Cary, NC).

In Situ Experiment

Animals, housing and diets. Six multi parity Holstein-Friesian dairy cows (192 ± 38 DIM; mean ± SD) fitted with a rumen fistula (Type 1C, Bar Diamond, Inc., Parma, Idaho, U.S.A) were used to assess the rate of fermentative degradation of feedstuffs fed to the animals in the earlier described passage experiment. At the start of the experiment animals had a BW of 550 ± 40 kg (mean ± SD) and produced 20.4 ± 1.7 kg (mean ± SD) FPCM/d. Animals were randomly distributed over 2 dietary treatments with diets based on a silage of higher digestibility (GSH) or lower digestibility (GSL) and allowed to adapt to their diets during a fortnight. During the experimental period the average daily DMI, which was offered in 4 equal portions at 0700, 1130, 1630 and 2100 h, was 14.5 ± 1.2 kg/d (mean ± SEM) with 48.9

± 4.6% (mean ± SEM) of the DMI as a commercial concentrate. Animals had free access to water and a commercial available mineral lick. Milking was done twice per day at 0700 and 1630 h. The experimental layout and handling of animals were submitted to and approved by an ethical committee, and executed in accordance with Dutch legislation on the use of experimental animals.

Treatments, sampling, and analyses. Frozen samples of the roughages (GSL, GSH) that were offered to the cows during the passage experiment were cut to a size of 0.5 to 1.0 cm using a paper cutter, homogenised, split into several portions and stored at –20°C in airtight plastic bags. The compound feed was ground to pass a 3-mm screen. Nylon bags (15 × 8-cm, pore size 40 μm, permeability 30%; PA 40/30, Nybolt, Switzerland) were filled with approximately 5 g DM of the feedstuffs. Bags filled with one of the two silage types were randomly distributed over incubation times (0, 2, 4, 8, 24, 48, 72 and 336 h), periods (3) and over the animals (2 × 3) receiving the same silage type (2). The bags with compound feed were randomly distributed over animals (6), incubation times (0, 2, 4, 8, 24, 48, 72 and 336 h) and periods (3). Incubations (2 to 72 h) were done according to the all-out procedure with a maximum of 23 bags per incubation cycle, where bags were retrieved from the rumen prior to the afternoon milking at 16.00 h. The undegradable fractions (U) were determined by 336-h incubations using 3 different animals. Sample disappearance with time was fitted using an exponential equation with inclusion of a lag time (*T*) (Robinson *et al.*, 1986), where the estimated undegradable fraction (*Fr*) was determined as the asymptotic value at t = ∞. Further details on incubation procedure and statistical analysis are described elsewhere Pellikaan *et al.* (2004[b]).

Results and Discussion

Marker Passage after Post-Ruminal Marker Introduction

Curve fit accuracy. Passage characteristics of Co, Cr and ^{13}CDM upon ileal and abomasal pulse dosing are summarised in Table 4, with the mean prediction error (MPE) indicating the goodness of fit. Curve fits of marker excretion patterns resulted in a model average for the root MSPE of 0.0142 g/kg and an average MPE of 22.8%. On average, about 94.6% of the MSPE was related to errors due to random disturbance, 2.6% to errors of central tendency and 2.8% to errors due to deviation of the regression slope from unity. Hence, the single exponential model gave a good description of all marker excretion patterns. The MPE showed no effect of marker (P = 0.290) and pulse dose site (P = 0.209), but did for cow (P = 0.048) and silage type (P = 0.021). The latter indicates that marker excretion patterns were fitted with higher accuracy for GSH based diets. The range of MPE-values observed in the current experiment is comparable to those observed by Pellikaan *et al.* (2004[c]), who reported respective MPE-values of 23% and 18% upon ileal and abomasal pulse dosing.

Fractional passage rates and residence times. The model estimates of *A* and *K*, and the calculated values for PCT and TMRT determined after ileal and abomasal marker introduction are also given in Table 4. The model main effects cow ($P < 0.001$), period ($P = 0.054$), pulse dose site ($P < 0.001$) and marker type ($P = 0.003$) contributed considerable to the variation of *K*, but silage type had no effect ($P = 0.732$). After ileal pulse dosing, *K* did not differ between marker and silage type ($P \geq 0.703$). Upon pulse dosing into the abomasum, Co gave higher values for *K* than Cr and ^{13}CDM, with the largest effect in the GSL group ($P \geq 0.137$). No significant differences in *K*-values between the silage types were observed within marker type ($P \geq 0.779$). Fractional marker passage was affected by site of pulse dose (ileum vs. abomasum), with lower *K*-values for Cr ($P = 0.223$) and ^{13}CDM ($P = 0.041$) after abomasal pulse dose in GSL fed animals. In GSH fed animals this effect became more pronounced for Co ($P = 0.139$), Cr ($P = 0.007$) and ^{13}CDM ($P = 0.001$). PCT and TMRT values determined after an ileal pulse dose were considerably lower than those obtained after an abomasal pulse dose ($P < 0.001$). Differences in PCT and TMRT related to silage type (GSL vs. GSH) were negligible ($P \geq 0.486$) for both pulse dose treatments (ileum vs. abomasum). No differences in PCT and TMRT between markers were found upon ileal pulse dosing ($P = 1.000$). After an abomasal pulse dose, the PCT of ^{13}CDM was reached at an earlier stage compared to Cr for both silage types (GSL, $P = 0.397$; GSH, $P \leq 0.001$), and for TMRT similar differences were observed. Values of *K*, PCT and TMRT are similar to those reported by Pellikaan *et al.* (2004[c]) where cows received a diet based on fresh grass. Their observed daily DMI of 14.9 kg was about 2.5 kg lower than the current experiment, but this appeared not to affect PCT and TMRT in the post-rumen compartments. This contrasts with earlier observations of Pellikaan *et al.* (2004[b,c]) and other observations (Huhtanen and Kukkonen, 1995; Mambrini and Peyraud, 1997; Pond *et al.*, 1998), which show a negative relationship between the TMRT and level of DMI. The silages used in the current experiment originated from a later cut but the same pasture than the grass used in the experiment of Pellikaan *et al.* (2004[c]), but the proportion of concentrate in the grass diet (24%) was considerably lower than in the current experiment (± 46%). As reviewed by Owens and Goetsch (1986) and Offer and Dixon (2000), the proportion of concentrate in the diet may considerably influence ruminal passage rate constants of the fluids and concentrate components and to a lesser extent that of roughage. This effect is likely not limited to the rumen compartment and also affects passage behavior through the post-rumen compartments. Therefore, the potential effect of level of feed intake to reduce the TMRT may have been counterbalanced by the increase in concentrate ratio, when comparing the current experiment with our earlier observations (Pellikaan *et al.*, 2004[c]). After marker introduction into the abomasum, the TMRT of Cr was significantly longer than that of Co and ^{13}CDM, suggesting a different passage behavior of this marker. Although ^{13}CDM did not differ in TMRT from Co, in combination with an earlier moment of PCT and a lower fractional passage rate also ^{13}CDM showed a different passage behavior than that of Co. The excretion patterns of the markers (not shown here) indicate more tailing of the particle phase markers compared to the

Table 4. Effect of silage type (GSL, GSH) on faecally determined passage characteristics following from pulse doses into the ileum and abomasum.[1,2]

	A		*K* (/h)		MPE (%)		PCT (h)		TMRT (h)		RP (%)	
	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH
Ileum pulse dose												
Co	1.02	2.29	0.404	0.419	24.3	18.0	3.84	3.94	6.51	6.36	69.2^{a}	73.3^{a}
Cr	1.09	1.62	0.354	0.369	33.7	22.7	3.43	3.51	6.46	6.28	101.4^{b}	101.4^{b}
^{13}CDM	0.94	1.49	0.390	0.469	26.2	23.4	3.44	3.50	6.21	5.86	58.9^{a}	65.7^{a}
Abomasum pulse dose												
Co	10.15^{a}	4.19	0.366	0.275	25.0	17.2	8.26	8.24^{ab}	11.25^{a}	12.14^{a}	70.5^{a}	74.8^{a}
Cr	3.53^{b}	1.62	0.221	0.168	26.0	21.8	8.52	10.03^{a}	14.45^{b}	16.30^{b}	93.6^{b}	85.8^{a}
^{13}CDM	1.08^{b}	1.27	0.220	0.212	24.1	12.5	6.97	6.77^{b}	12.36^{ab}	12.03^{a}	46.1^{c}	36.8^{b}
Model parameters												
SEM		1.42		0.034		4.9		0.45		0.59		3.6
rMSE		3.39		0.082		11.8		1.08		1.41		8.6
Cow (I)		NS		***		*		†		***		NS
Period (II)		†		†		NS		NS		†		**
Site (III)		**		***		NS		***		***		***
Marker (IV)		**		**		NS		**		***		***
Silage (V)		NS		NS		*		NS		NS		NS
I × IV		NS		NS		NS		NS		NS		NS
II × IV		NS		NS		NS		NS		NS		NS
III × IV		*		*		NS		**		***		***
V × IV		NS		NS		NS		NS		NS		NS
V × III × IV		NS		NS		NS		NS		NS		NS

[1] *A* = initial marker concentration where t = 0 corresponds with the time when peak concentration is reached (Co and Cr in g/kg, ^{13}C in atom percentage excess (% × 10)); *K* = fractional passage rate (/h) estimated from the declining line [Eq.1]; MPE = mean prediction error; PCT = moment when peak concentration is reached; TMRT = 1/*K* + PCT; RP = marker recovery relative to the amount pulse dosed (%).

[2] GSL = grass silage of low digestibility, GSH = grass silage of high digestibility.

[3] Statistical model includes main effects cow (3), period (4), site of pulse dose (2), marker (3), silage type (2) and the interaction terms where marker effect is included. Remaining interaction terms not included in the Table. SEM = standard error of the mean; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[a,b,c] Within site of pulse dose and silage type mean values differ between markers ($P < 0.05$).

liquid phase marker, and hence, confirming this difference in passage behavior between liquid and particle phase. This is consistent with our earlier observations (Pellikaan *et al.*, 2004[b,c]) and agrees with the observations of O'Connor *et al.* (1984) who suggested that particle phase markers were likely to endure more sequestration in the small intestine and in the caecal-large intestine compartment compared to liquid phase markers.

Marker recovery. Recovery (RP) of Cr upon ileal pulse dosing was complete, but significantly different from Co and ^{13}CDM ($P < 0.001$), that also differed significantly from 100% RP ($P < 0.001$). After an abomasal pulse dose the RP of Cr tended to differ from 100% in GSL fed animals ($P = 0.084$) but was significantly lower for GSH ($P = 0.003$). Although the RP of Co upon abomasal pulse dosing was again lower than Cr ($0.002 \leq P \leq 0.561$), no further decrease was observed compared to ileal Co recovery. Part of this lower recovery can be attributed to loss of marker through uptake via the intestinal wall as reported in literature (Udén *et al.*, 1980). However, they do not fully account for the 25 to 30% loss of Co currently observed, and other sources of variance (e.g., pulse dosing, reflux into the intestines or omasum, faecal sampling, laboratory analyses, etc.) contributed to this. Ileal recovery of ^{13}CDM suggested an apparent large intestinal digestibility for GSL and GSH of respectively 41.1 and 34.3%. The apparent digestibility of ^{13}CDM after introduction into the abomasum was non-significantly higher in case of GSL (53.9%; $P = 0.328$) but distinctly higher for GSH (63.2%; $P < 0.001$). This suggests that about 10% of the GSL-labelled material and 25 to 30% of the GSH-labelled material was digested in the small intestine compartment. This agrees with our earlier observations where animals were kept on a grass silage based diet at low level of intake (Pellikaan *et al.*, 2004[b]). Here the recovery of ^{13}C in the DM showed a 12% loss of marker in the small intestine, 25% with respect to higher digestible non cell wall fraction (^{13}CNDS) and no loss of cell walls (^{13}CNDR). In the current experiment, the combination of a high N supply to the pasture and an early moment of harvest for GSH resulted in a silage of higher digestibility and higher CP/NDF ratio that likely contributed to the difference in small intestinal DM digestibility between the two silages. Although no quantitative information is available on the NDR and NDS fractions in the current experiment, based on earlier experience it can be hypothesised that the larger part of this ^{13}CDM digestion is associated with the disappearance of the NDS fraction. However, in contrast to these findings no differences in RP-values were found between ileal and abomasal pulse dosed ^{13}C-labelled materials in cows receiving grass silage and/or fresh grass close to *ad libitum* (Pellikaan *et al.*, 2004[b,c]). From this data it appears that no substantial digestion of marker occurs in the small intestine compartment. This shows that the information obtained in our earlier work and in the current experiment do not reveal a consistent pattern, but the results are also based on a limited number of observations. However, the use of ^{13}C-labelled material demonstrates the potential of this marker to acquire quantitative and qualitative information on hindgut passage dynamics of feed components in relation to the digestion in the lower tract.

Marker Passage after Ruminal Marker Introduction

Curve fit accuracy. Model estimates describing the faecal and ileal marker excretion patterns (A, K_1, K_2, N) and the accuracy of curve fitting (MPE) are summarised in Table 5. Curve fits resulted in a model mean value for the root MSPE of 0.0089 g/kg and a MPE of 12.5%, of which 96.6% was related to errors due to random disturbance, 1.6% to errors of central tendency and 1.8% to errors due to regression. This indicates that the multi-compartmental model in general gave accurate curve fits. The main effects sample site (P = 0.404) and silage type (P = 0.672) did not affect MPE, but cow (P = 0.003) and marker (P < 0.001) significantly contributed to the variance in MPE. The highest average MPE was obtained for ^{13}CNDR (15.4%), and differed significantly from Cr (9.7%, P = 0.001) and Co (11.2%, P = 0.029). In case of Co 4.4% of the MSPE contributed to errors of central tendency, which was higher than that of Cr (1.5%), ^{13}CDM (0.8%), ^{13}CNDR (0.6%) and ^{13}CNDS (1.0%) (P < 0.001). Errors due to regression did not differ between markers, but errors of random disturbance were lower for Co (94.1%) compared to all other markers ($P \leq$ 0.019). The range of the MPE in the current experiment is similar to our earlier observations (Pellikaan *et al.* 2004[c]), who reported MPE-values for faecal and ileal determined excretion curves of 10% and 15% respectively.

Fractional passage rates and estimated compartments. The model estimates of A, K_1, K_2 and N determined from faecal and ileal marker excretion patterns are also in Table 5. The model main effects marker type (P < 0.001) and silage type (P = 0.001) contributed largely to the variation of K_1, whilst cow (P = 0.981), period (P = 0.890) and sample site (P = 0.159) appeared to have no effect. In case of K_2, model main effects sample site (P < 0.001), marker type (P < 0.001) and silage type (P = 0.008) were contributing factors. In all cases the K_1 and K_2-values for Co were significantly higher compared to the other markers (P < 0.001). This is in line with the expectation and agrees with earlier observations (Pellikaan *et al.*, 2004[b,c]). The faecally determined fractional passage rates of Cr (K_1 ± 0.054/h; K_2 ± 0.290/h) are considerably higher than reported in literature (e.g., Dhanoa *et al.*, 1985, 0.026 and 0.179/h; Beauchemin and Buchanan-Smith, 1989, 0.026 and 0.171/h; Thiago *et al.*, 1992, 0.027 and 0.138/h). Differences in marker particle size between literature and our observations likely form the explaining factor, as demonstrated by Bruining and Bosch (1992) and Egan and Doyle (1984). The K_1-values of the internal markers were lower than those of Cr and for K_2 this effect was even more profound, with the largest contrasts between Cr and ^{13}CNDR (0.001 > $P \leq$ 0.698). From the internal markers, ^{13}CNDS had the highest K_1-values. Differences between markers as well as their absolute values for K_1 were comparable with our earlier observations (Pellikaan at al., 2004[b,c]).

The number of compartments (N) estimated for external markers was considerably higher than that of the internal markers. However, in 3 occasions (1 for Cr and 2 for Co) the estimations of N were excessively high ($N \geq$ 869) and hence, were treated as outliers. In an experiment with grass silage fed dairy cows, Pellikaan *et al.* (2004[b]) observed faecally determined values of N ranging from 14 to 167, and ileal determined N-values ranging from 7

Table 5. Effect of silage type (GSL, GSH) on faecal and ileal determined passage characteristics following from ruminal marker introduction.[1,2]

	A		*K*$_1$ (/h)		*K*$_2$ (/h)		*N*		MPE (%)	
	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH
Faecal excretion[4]										
Co	4.546	15.681^{a**}	0.105^{a}	0.133^{a*}	0.531^{a}	0.613^{a}	51.2^{a}	47.8	12.0	14.4
Cr	3.069	3.126^{b}	0.054^{b}	0.053^{b}	0.249^{b}	0.331^{b}	25.7^{ab}	39.6	9.5	8.0
^{13}CDM	1.284	1.665^{b}	0.030^{bc}	0.036^{bc}	0.118^{b}	0.150^{c}	7.9^{b}	11.1	11.9	10.6
^{13}CNDR	0.637	1.160^{b}	0.017^{c}	0.020^{c}	0.107^{b}	0.091^{c}	12.1^{b}	7.8	14.4	14.6
^{13}CNDS	6.385	5.152^{b}	0.040^{b}	0.049^{b}	0.120^{b}	0.148^{c}	8.0^{b}	10.6	13.7	12.3
Ileal excretion[4]										
Co	1.510	6.529	0.112^{a}	0.157^{a***}	0.957^{a}	0.849^{a}	19.2	48.5^{a}	11.1	7.5^{a}
Cr	1.668	1.694	0.052^{b}	0.049^{b}	0.333^{b}	0.566^{b***}	19.6	38.9^{ab}	9.3	12.1^{ab}
^{13}CDM	2.245	1.113	0.033^{bc}	0.034^{b}	0.147^{c}	0.180^{c}	6.8	5.6^{b}	12.8	13.2^{ab}
^{13}CNDR	0.456	2.340	0.016^{c}	0.034^{b}	0.122^{c}	0.117^{c}	12.1	6.0^{b}	13.2	19.3^{b}
^{13}CNDS	3.109	0.854	0.041^{b}	0.038^{b}	0.131^{c}	0.174^{c}	6.6	5.4^{b}	15.1	15.1^{ab}
Model parameters[3]										
SEM		1.673		0.005		0.031		6.9		2.0
rMSE		4.019		0.011		0.075		16.5		4.8
Cow (I)		NS		NS		NS		NS		**
Period (II)		NS		NS		NS		NS		NS
Sample (III)		**		NS		***		†		NS
Marker (IV)		***		***		***		***		***
Silage (V)		†		***		**		NS		NS
I × IV		NS		*		*		†		NS
II × IV		NS		NS		***		NS		NS
III × IV		*		*		***		NS		NS
V × IV		**		***		**		NS		NS
V × IV × III		NS		NS		**		NS		NS

[1] *A* = initial value of marker concentration (Co and Cr in g/kg, ^{13}C in atom percentage excess (% × 1000)); *K*$_1$ = fractional passage rate (/h) from the slowest compartment; *K*$_2$ = fractional passage rate (/h) from the second slowest compartment; *N* = number of compartments [Eq.2]; MPE = root of the mean squared prediction error (MSPE) proportionally to the observed mean;
[2] GSL = grass silage of low digestibility, GSH = grass silage of high digestibility.
[3] Statistical model includes main effects cow (3), period (4), sampling site (2), marker (3), silage type (2) and the interaction terms where marker effect is included. Remaining interaction terms not included in the Table. SEM = standard error of the mean; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.
[4] Within sampling site (Faecal or Ileal) and silage type (GSL or GSH) different superscripts indicate significant differences between markers ($P < 0.05$); Within sampling site the mean values of markers differ between silages, † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

to 54. In fresh grass fed dairy cows they (Pellikaan *et al.*, 2004[c]) observed extremely high estimates for the ileally determined *N*. In both studies also extremely high values for K_2 were reported, always in combination with the external markers, and always associated with ileal excretion patterns. They attributed both effects to an insufficient number of measurement points before markers reached PCT. To circumvent this problem, more samples were taken before the expected moment of PCT in the current experiment.

Testing within sample site (faecal or ileal) for differences in fractional passage rates between silage treatments (GSL vs. GSH) shows that GSH based diets gave in general higher values for K_1 ($P \leq 0.023$) and K_2 ($P \geq 0.194$). The differences in K_1 between silages at both sample sites were only significant for Co ($P \leq 0.013$). In case of K_2, only the ileal determined passage rates of Cr gave significant differences between silages ($P \leq 0.001$). By scaling the differences in rate constants between silages of external markers (e.g., for K_1 of Co; GSH, 0.133/h vs. GSL, 0.105/h), fractional passage rates of external markers are 21% lower for GSL. The faecally determined K_1 and K_2 values, of ^{13}CDM and ^{13}CNDS respond with a similar 15 to 21% decrease when changing from GSH to GSL treatment. However, ileal ^{13}C-excretion patterns were not consistent with the faecally observed differences, which may be explained by inaccuracies associated with chyme collection. The differences in K_1-values between silages agree with observations of Bosch *et al.* (1992) and Gasa *et al.* (1991) who both assigned dairy cows to grass silages harvested at different stages of maturity (early vs. late) combined with different levels of concentrate in the diets. Bosch *et al.* (1992) observed significant positive relations between silage NDF content and the fractional passage rate of Cr-NDF and tritium labelled hay, in cows receiving low concentrate diets. The high concentrate diets gave a similar response, but here the effect was only significant for Cr. In contrast, the fractional passage rates of Co tended to decrease when silage NDF content increased. Gasa *et al.* (1991) obtained significantly higher faecally determined fractional passage rates for Cr mordanted faecal particles in dairy cows receiving more mature grass silage. The fractional passage of Ytterbium labelled silage showed a similar but non-significant response to silage maturity. These data and our observations suggest that in order to quantify the effects of silage quality on fractional particle passage using external markers, the type of fiber source and method or mode of marker adhesion are important factors that will largely determine the outcome of experimental results.

Compartmental residence times. Table 6 presents the values obtained for PCT, TT, CMRT1 ($1/K_1$),.CMRT2 ($1/K_2$), TMRT and RP. The faecally and ileally determined PCT and TT of particle markers occurred at an earlier stage in the GSH group compared to GSL, with largest silage effect observed in PCT for ^{13}CDM ($P \leq 0.010$) and ^{13}CNDS ($P \leq 0.059$). In contrast, the liquid phase marker Co tended to higher PCT and TT-values in GSH fed animals. Sampling site (faecal vs. ileal) had a significant effect on PCT for all markers ($P \leq 0.019$) and showed a similar but less profound response for TT. The values for PCT and TT are in line with our earlier observations (Pellikaan *et al.*, 2004[b,c]). Beauchemin and Buchanan-Smith (1989) observed values for TT for Cr mordanted silage (14.6 h) and hay

Table 6. Effect of silage type (GSL, GSH) on faecal and ileal determined moment of peak concentration (PCT), the transit time (TT), the compartmental residence times ($CMRT_1$, $CMRT_2$), the total tract mean residence time (TMRT) and the marker recovery (RP) after ruminal marker introduction.[1,2]

	PCT (h)		TT (h)		$CMRT_1$ (h)		$CMRT_2$ (h)		TMRT (h)		RP (%)	
	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH	GSL	GSH
Faecal excretion												
Co	12.23^{a}	13.23^{a}	7.68^{a}	9.08^{a}	9.13^{a}	8.19^{a}	1.97^{a}	1.86^{a}	18.77^{a}	19.13^{a}	74.9^{a}	71.0^{a}
Cr	22.35^{b}	20.17^{b}	12.94^{ab}	12.01^{a}	18.46^{ab}	19.83^{a}	4.14^{a}	3.17^{a}	35.53^{ab}	35.01^{ab}	104.9^{b}	102.2^{b}
^{13}CDM	33.09^{c}	27.80^{c†}	15.43^{b}	13.63^{ab}	34.74^{b}	28.80^{a}	8.91^{b}	6.86^{b}	59.07^{c}	49.29^{b}	21.4^{cd}	19.8^{cd}
^{13}CNDR	46.14^{d}	42.95^{d}	20.46^{bc}	18.98^{b}	69.52^{c}	57.16^{b}	10.50^{b}	11.00^{c}	100.48^{d}	87.14^{c}	12.3^{c}	10.9^{c}
^{13}CNDS	31.24^{c}	27.23^{c}	17.22^{b}	15.26^{ab}	27.45^{ab}	22.04^{a}	8.71^{b}	6.82^{b}	53.39^{bc}	44.13^{b}	29.5^{d}	26.2^{d}
Ileal excretion												
Co	5.69^{a}	7.38^{a}	2.56^{a}	4.61^{a}	9.61^{a}	5.86^{a}	0.93^{a}	1.32^{a}	13.10^{a}	11.78^{a}	-	-
Cr	15.74^{b}	11.24^{a}	7.63^{ab}	5.71^{a}	20.23^{a}	20.57^{a}	3.03^{a}	2.11^{a}	30.89^{ab}	28.39^{ab}	-	-
^{13}CDM	26.09^{c}	19.76^{b*}	11.59^{b}	7.16^{a}	34.21^{a}	30.19^{a}	7.78^{b}	5.96^{b}	53.58^{c}	43.31^{b}	-	-
^{13}CNDR	36.54^{d}	32.06^{c}	13.95^{bc}	15.51^{b}	61.63^{b}	41.68^{b}	8.94^{b}	9.22^{c}	84.52^{d}	66.41^{c}	-	-
^{13}CNDS	24.67^{c}	19.22^{b†}	11.11^{b}	6.72^{a}	26.36^{a}	26.60^{a}	7.67^{b}	5.88^{b}	45.14^{bc}	39.20^{b}	-	-
Model parameters[3]												
SEM	1.04		1.22		4.77		0.55		4.28		2.9	
rMSE	2.51		2.92		11.46		1.33		10.28		6.9	
Cow (I)	***		**		NS		***		NS		NS	
Period (II)	**		NS		NS		NS		NS		NS	
Sample (III)	***		***		NS		***		***		NS	
Marker (IV)	***		***		***		***		***		***	
Silage (V)	***		*		*		**		**		NS	
I × IV	***		**		*		**		*		NS	
II × IV	NS		**		**		†		†		NS	
III × IV	†		NS		NS		NS		NS		NS	
V × IV	***		*		NS		*		NS		NS	
V × IV × III	NS		NS		NS		NS		NS		NS	

[1] $CMRT_1 = 1/K_1$, retention time for the slowest compartment (h) and $CMRT_2 = 1/K_2$, retention time for the second slowest compartment (h); TT = time of first marker appearance or transit time (h); TMRT = $CMRT_1 + CMRT_2$ + TT (h); RP = marker recovery relative to the amount pulse dosed (%) and corrected for losses through ileal chyme sampling.

[2] GSL = grass silage of low digestibility, GSH = grass silage of high digestibility.

[3] Statistical model as descibed in Table 5; SEM = standard error of the mean; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[4] Different superscripts indicate significant differences between markers within sampling site (Faecal or Ileal) and silage type (GSL or GSH) ($P < 0.05$); Differences between silages (GSL vs GSH) within sampling site are indicated by, † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

(14.9 h), and Ytterbium labelled silage (17.9 h), based on the multi-compartmental model estimations. Using the same model and Cr mordanted hay, Thiago *et al.* (1992) observed distinctly higher values for TT (20.5 to 23.6 h), which could be explained by their reported 30% lower level of daily DMI compared to our observed DMI.

The CMRT1 and CMRT2 of the ^{13}C-labelled fractions are longer than those of Cr and Co. In combination with a delay in TT this results in significantly longer TMRT. In general the TMRT-values of Co and Cr appeared to be similar to earlier observations in cows fed grass silage close to *ad libitum* (Pellikaan *et al.*, 2004[b]) and in cows receiving fresh grass (Pellikaan *et al.*, 2004[c]). Within the current experiment, the difference in roughage quality did not affect the TMRT of the external markers used here. Differences in TMRT between GSH and GSL for ^{13}CDM (9.8 h), ^{13}CNDR (13.3 h) and ^{13}CNDS (9.3 h) are larger compared to Co and Cr, but remain non-significant ($P \geq 0.295$). With ^{13}CDM and ^{13}CNDS about 40% of the difference in TMRT between silages was related to a changed CMRT2 and TT. This suggests that events associated with these compartments can considerably affect the passage behavior of these fractions. In case of ^{13}CNDR more than 90% of this difference was attributable to CMRT1, indicating that the other compartments only have a minor effect on TMRT when changing from GSH to GSL.

The ^{13}C-markers used in the current trial are incorporated in the different plant fractions. The rate at which these labelled materials escape from the rumen is determined by the rate of particle size reduction on the one hand and the change in functional specific gravity (FSG) through rumination on the other (Offer and Dixon, 2000). It is likely that the less mature GSH is more easily affected by these processes and prone to escape the rumen at an earlier stage than the GSL-material. Although not significant, from our data (Table 6) it appears that the ^{13}C-markers are able to distinguish this dietary treatment effect. In contrast, Cr-NDF particles (mean size 0.5 mm) do not undergo the process of degradation, and their FSG will not be affected by fermentative processes like gas entrapment. Therefore, any changes in passage behavior of Cr-NDF are primarily related to the physical constitution of the rumen content and its filtering capacity. However, in spite of the qualitative contrast between GSH and GSL, and its likely effect on the rumen content, the passage of Cr from the rumen was not affected. Therefore, the internal marker ^{13}C seems to be superior to the external particle phase marker.

Marker recovery. The RP of Cr upon introduction in the rumen was complete in all cases (Table 6). However, the RP-values for Co were considerably lower than those of Cr ($P < 0.001$) and similar to the partial marker loss described earlier. The RP-values of ^{13}CDM, ^{13}CNDR and ^{13}CNDS were significantly lower that those of Cr and Co, suggesting that a large part of these labelled fractions were digested. Although ^{13}C-labelled marker recovery had slightly lower values in the GSH treatment, indicating higher digestibility of the markers in this diet, differences between the silage treatments were negligible ($P \geq 0.998$). The overall digestibility of ^{13}CDM in the GSL group (78.6%; 100 - RP) was slightly lower than that of the GSH (80.2%), and was in line with earlier observations reported elsewhere (Pellikaan *et al.*, 2004[b,c]). The high digestibility of ^{13}CNDR reflects a true digestibility coefficient for this

fraction, as endogenous components do not interfere. With respect to the ^{13}C-labelled DM and NDS fractions, endogenous components are likely to affect these fractions and therefore, should be regarded as apparent digestibility coefficients. The apparent digestibility of ^{13}CNDS is being derived from the difference in cumulative recovery of ^{13}CMD and ^{13}CNDR. Therefore, the endogenous component will have a more pronounced impact on the ^{13}CNDS-fraction, resulting in the higher RP-values observed for this fraction.

Rumen Fermentation

In situ degradation. Table 7 summarises the DM, OM, NDF and CP degradation characteristics for both silages and the specially fabricated compound feed. The fractional degradation rates (K_d) for GSH were in all cases higher then GSL, with relative differences of about 19% for DM and OM, 13% for NDF and 38% for CP. Valk *et al.* (1996) studied the effects of varying management conditions for perennial rye grass on its *in situ* degradation characteristics. Their observations support the differences obtained in the current experiment, but their reported K_d-values for CP were considerably higher than our observations, which is also the case for studies of Tóthi (2003), De Visser *et al.* (1993) and Bosch (1991). The latter evaluated 4 grass silages differing in cell wall content and digestibility for their *in situ* degradation and observed significant lower K_d-values for the DM (6.74 vs. 4.39%/h) and NDF-fraction (6.35 vs. 3.99%/h) in the silage with a higher cell wall content and lower digestibility. Interestingly, the contrasts in NDF and CP content between the GSL and GSH used in the current experiment were even larger than those reported by Bosch (1991), but this was not reflected in K_d-values of the DM, OM and NDF fractions in the current experiment. Rinne *et al.* (1997) reported a similar negative relation between the K_d for NDF (ranging from 2.7 to 4.4%/h) and the level of NDF (ranging from 409 to 625 g/kg DM). Valk *et al.* (1996) suggested a linear relation between the size of the undegradable NDF-fraction and the height of fractional degradation rate of the NDF (where $K_d = 9.47 - 0.4815 \times U$; $r^2 = 0.75$). However, applying our data to the equation shows that our data does not agree with their findings.

The fractional degradation rates of the compound feed tended to be higher in the GSL-fed animals. This implies that a smaller amount escapes from rumen degradation when assuming fixed fractional rate constants for passage. The GSL-fed animal showed to increase the rumen residence times of labelled silage. If the concentrate part of the diet in animals fed GSL is also retained longer, the amount of concentrate material escaping rumen degradation might be reduced as well. However, *in situ* incubated materials are not subjected to passage and a difference in degradation rate is attributable to the efficiency in rumen functioning to degrade a certain component. Animals receiving more fibrous diets are likely to adapt their rumen microbial system with a preference toward fibrolytic degradation, and hence, rather depress concentrate degradation than increase it as observed in the current experiment. However, part of the higher degradation rate may be explained by the composition of the compound feed,

Table 7. *In situ* rumen degradation characteristics of three feeds, a compound feed (CF), and two grass silages (GSL, GSH) in cows receiving either GSL or GSH based diets.[1,2]

Dietary treatment	GSL		GSH	
Feeds	CF	GSL	CF	GSH
	---------- DM fraction ----------			
W, (%)	44.6	25.5	44.6	32.2
D, (%)	48.2	56.7	48.2	55.5
U, (%)	7.2	17.8	7.2	12.4
Fr, (%)	7.7	17.9	6.8	13.1
T, (h)	0.27	3.18	2.74	1.19
Kd, (/h)	0.051	0.026	0.044	0.032
	---------- OM fraction ----------			
W, (%)	43.2	19.9	43.2	28.0
D, (%)	50.0	62.2	50.0	59.4
U, (%)	6.8	17.9	6.8	12.6
Fr, (%)	7.2	17.9	6.2	13.1
T, (h)	3.10	3.47	0.89	0.87
Kd, (/h)	0.051	0.026	0.043	0.032
	---------- NDF fraction ----------			
W, (%)	5.4	0.0	5.4	0.0
D, (%)	76.6	78.5	76.6	81.4
U, (%)	17.9	21.5	17.9	18.6
Fr, (%)	17.4	21.3	16.2	18.8
T, (h)	5.04	6.71	2.13	6.02
Kd, (/h)	0.047	0.026	0.041	0.030
	---------- CP fraction ----------			
W, (%)	40.9	47.7	40.9	58.0
D, (%)	54.1	35.7	54.1	35.3
U, (%)	5.0	16.6	5.0	6.8
Fr, (%)	5.3	17.1	4.0	8.7
T, (h)	2.10	0.48	2.83	0.00
Kd, (/h)	0.029	0.029	0.028	0.047

[1] W = washable fraction; D = insoluble but potentially degradable fraction, 100 – W – U; U = undegradable fraction determined after 336 h of incubation; *Fr* = undegradable fraction derived from the asymptote of the degradation curve at t = ∞ h; Fractions are expressed as percentage of the weighted sample; K_d = fractional degradation rate (/h); *T* = lag time (h).
[2] GSL = grass silage of low digestibility, GSH = grass silage of high digestibility.

which gives a relatively high amount of cell walls. A possible adaptation in rumen functioning was not supported by any change in the fermentative end products (Table 8).

Fermentation end products. Although with both diets animals were fed to meet their requirements for energy (NEL) and intestinal degradable protein (DVE), the GSL-fed animals had a negative degradable protein balance (OEB), indicating a shortage of N relative to the energy availability in the rumen. An imbalance between N and energy availability may lead to

Table 8. The effect of silage type and time after feeding on the chemical composition of the rumen liquid.[1,2]

	pH	Acetic mM	Propionic mM	Buteric mM	Valeric mM	Branched mM	TVFA mM	NH_3 mM	NH_3:TVFA mM/mM
GSL									
T0	6.18[a]	85.41[a]	21.91[a]	16.29[a]	1.54[a]	1.22[a]	126.38[a]	3.55[a]	0.028[a]
T30	6.13[ab]	86.07[ab]	24.09[b]	17.11[a]	1.71[b]	1.46[b]	130.46[a]	6.41[b]	0.049[b]
T90	6.09[b]	89.70[b]	25.66[c]	18.25[b]	1.98[c]	1.62[c]	137.21[b]	7.97[c]	0.058[c]
GSH									
T0	6.14[a]	87.47[a]	23.83[a]	18.28[a]	1.76[a]	1.88[a]	133.21[a]	5.01[a]	0.038[a]
T30	6.11[ab]	89.07[a]	26.80[b]	19.26[b]	1.96[b]	2.26[b]	139.22[b]	10.79[b]	0.076[b]
T90	6.08[b]	93.46[b]	29.63[c]	21.77[c]	2.38[c]	2.77[c]	150.01[c]	13.34[c]	0.089[c]
Model parameters[3]									
SEM	0.02	1.21	0.38	0.30	0.05	0.05	1.77	0.29	0.002
rMSE, main plots	0.01	4.46	3.18	1.51	0.02	0.05	12.12	0.29	1.2E-5
rMSE, sub plots	0.01	16.51	1.54	1.01	0.02	0.03	38.12	0.82	5.3E-5
Silage	NS	NS	*	*	*	**	*	***	***
Cow	NS	NS	NS	NS	NS	NS	NS	*	†
Period	NS	NS	NS	NS	NS	NS	NS	†	*
FT	***	NS	**	†	NS	***	NS	†	†
TAF	***	***	***	***	***	***	***	***	***
FT × TAF	†	NS	NS	NS	NS	NS	NS	NS	NS
Silage × TAF	NS	NS	***	***	*	***	†	***	***

[1] T0 = time (t = 0) at feeding roughage; T30 = time (t = 30 min) at feeding compound feed; T90 = time (t = 90 min) after the roughage feeding; Branched = iso-buteric + iso-valeric acid; TVFA = acetic + propionic + buteric + valeric + branched.

[2] GSL = grass silage of low digestibility, GSH = grass silage of high digestibility.

[3] Statistical model includes silage (2), cow (3) and period (4) as main plot effects tested against the interaction term (silage × cow × period), and feeding time (FT, 5) and time after feeding (TAF, 3) as subplot effects with interaction terms tested against the model error term. SEM = standard error of the mean; rMSE = root mean square error; † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[a,b,c,d] Within columns mean values differ ($P < 0.05$).

impaired microbial protein synthesis (Tamminga *et al.*, 1994). This is further supported by the difference in ruminal NH_3 concentrations between the two diets (Table 8). Rumen samples collected before feeding (T0) showed NH_3 levels of 3.55 and 5.01 mM ($P \geq 0.010$) for GSL and GSH, respectively. For an optimal rumen functioning the NH_3 concentration should average 50 to 160 mg/L (2.94 to 9.41 mM; Hume *et al.* 1970; Roffler *et al.*, 1974; Satter & Slyter 1974). With NH_3 concentrations at time T0 in the GSL group ranging from 1.65 to 5.24 mM with 12 out of 24 observations below 3.00 mM, it appears that NH_3 concentrations before feeding may have had a negative effect on microbial activity. Ruminal total VFA (TVFA) concentrations in GSL based diets increased with time after feeding (T0 to T30, $P = 0.219$; T30 to T90, $P < 0.038$). In GSH based diets the effects became more pronounced ($P \leq 0.020$). At T0 the TVFA for GSH did not significantly differ from GSL ($P = 0.152$), but the effect became more pronounced after roughage (T30; $P = 0.032$) and concentrate feeding (T90; $P < 0.001$). Highest values for TVFA were observed 1 h after concentrate feeding (T90) in the GSH group, with in 50% of the occasions TVFA values between 150 to 170 mM in combination with high levels of NH_3 (10.5 to 15.5 mM). However, this did not seem to affect ruminal pH, and regression of TVFA on ruminal pH and TVFA gave low correlations (GSL, pH = 6.85 – 0.006·VFA, r = -0.42; GSH, 6.91 – 0.006·VFA, r = -0.48), which considerably differ from the Eq. (pH = 7.73 – 0.014·VFA) as reported by Tamminga and Van Vuuren (1988). The ratio between ruminal NH_3 and TVFA increased significantly for both GSL and GSH after roughage and concentrate feeding. However, no relations were observed between ruminal pH and NH_3:TVFA (GSL, $r^2 = -0.31$; GSH, $r^2 = -0.01$). Therefore, it may be concluded that although differences in silages were considerable and appeared to affect TVFA and VFA patterns as well as NH_3, this did not alter ruminal pH, and hence, gives no indication of a depression in rumen functioning.

Compartmentalisation of the Gastro-Intestinal Tract

The introduction of markers at various locations into the gastro-intestinal tract (GI-tract) and the faecal and ileal excretion patterns of these markers, allows to physical compartmentalisation of the GI-tract into several segments (Figure 1). The marker residence times in these segments were obtained by combining the data from Table 4 and 6, which were then used for comparison with the TT, CMRT1, CMRT2 and TMRT as estimated by the multi-compartmental model (MC). The large intestinal residence time ($CMRT_{LI}$) can be indirectly calculated from the difference in faecally and ileally determined TMRT (Table 6; Figure 1, A - D), and compared with the TMRT observed after ileal pulse dosing ($TMRT_{IL}$) (Table 4). The indirectly determined $CMRT_{LI}$ of markers averaged 5.3 h for GSL and 6.7 h for GSH, which were in line with the directly measured $TMRT_{IL}$ of 6.4 and 6.2 h, respectively. One of the model assumptions for MC is that the second slowest compartment (CMRT2) represents the caecum/ large intestine compartment. Comparison of the faecally determined CMRT2 with the $TMRT_{IL}$, shows that the CMRT2 of Co and Cr tend to

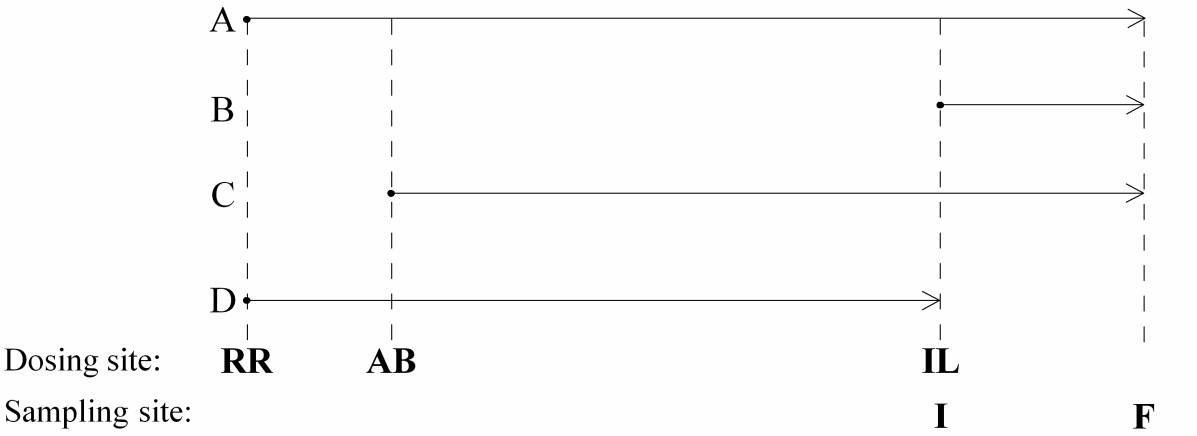

Figure 1. Schematic representation of the GI-tract where RR, AB and IL respectively depict the ruminal, abomasal and ileal pulse dose sites. Samples were collected from the ileum (I) and faeces (F). A= total GIT, B = large intestine, C = post rumen, D = total GIT minus caecum/large intestine, A – C = rumen, A – D = caecum/large intestine, B – C = abomasum/small intestine

underestimate the $TMRT_{IL}$ with respective averages of 4.5 and 2.5 h, whilst ^{13}CDM gives an average overestimation of about 2 h. The underestimation of CMRT2 for Co and Cr was also observed in earlier work (Pellikaan *et al.*, 2004[b,c]) and is likely related to inaccuracies in curve fitting, as discussed in an earlier section of this paper.

By subtracting the TMRT after abomasal pulse dosing ($TMRT_{AB}$) from the faecally determined TMRT after rumen pulse dosing, the residence time in the reticulo-rumen and omasum is obtained. Differences between the latter and the faecally determined CMRT1 should give information on the omasal residence time ($CMRT_{OM}$). The $CMRT_{OM}$ of Co gives negative values for both silage treatments (≈ -1.5 h), indicating that the liquid phase is passing directly on to the abomasum. In case of Cr the $CMRT_{OM}$ of GSL and GSH were respectively 2.5 and -1.1 h, suggesting that Cr particles in both diets also pass rather quickly. The $CMRT_{OM}$ of ^{13}CDM in GSH fed animals was 8.5 h and for GSL based diets 12.0 h. This indicates that the ^{13}C-labelled GSL is considerably longer retained in the omasum. Our earlier observations (Pellikaan *et al.*, 2004[b,c]) support the differences between markers as observed in the current experiment.

The small intestinal residence time ($CMRT_{SI}$) is calculated from the difference in $TMRT_{AB}$ and $TMRT_{IL}$. (Figure 1; B - C). The faecally determined TT (TT_F) (Table 6) is assumed to reflect the time required for longitudinal propulsion of digesta through the tubular sections. However, Pellikaan *et al.* (2004[c]) reported that TT_F overestimates the $CMRT_{SI}$, which is in line with the current observations where Co and Cr give an average 3-h overestimation and ^{13}CDM about 9 h. Excluding the influence of the large intestine (i.e. comparing the ileal derived TT_C-value with $CMRT_{SI}$) resulted for Co and Cr in negative values and for ^{13}CDM in positive values (GSL, 5 h; GSH, 1h). Therefore, it appears that both TT_F and TT_C are not solely attributable to a time delay caused by the intestines, but that a variable part is associated with a non-mixing delay within the mixing compartments, as proposed by Pellikaan *et al.* 2004[c]).

Marker Digestibility in Relation to Passage and Degradation

Introduction of ^{13}C-labelled GSL and GSH along the GI-tract shows a general decrease in marker recovery with highest values obtained after introduction into the ileum (58.9 and 65.7% respectively), followed by RP after abomasal pulse dosing (46.1 vs. 36.8%) and ruminal pulse dosing (21.4 vs. 19.8%). In combination with the corresponding TMRT-values these results give information on the fractional disappearance of marker throughout the GI-tract. The relation between RP-values of ^{13}CDM and their corresponding TMRT-values was determined using the model described by Robinson *et al.* (1986) without inclusion of a lag time (Figure 2). The undigestible (U) fractions for GSL and GSH estimate 20.7 and 18.6% respectively (Figure 2), and overestimate the *in situ* determined U-fractions of the DM and OM (Table 7). However, the data match well with the U-fractions of NDF for GSL (21.3%) and GSH (18.8%). GSL appeared to have a water soluble fraction (W) fraction of 16.4% and an estimated D-fraction of 62.9%, whilst in GSH these fractions could not be distinctly separated (W = 0%, D= 81.5%; Figure 2). The fractional rate constants describing the decrease in ^{13}C-labelled GSL and GSH with time were 0.073/h and 0.115/h respectively. In theory, this fractional rate constant (K_{tc}) represents the marker disappearance from the GI-tract through processes of fermentation and digestion. For example, after correcting the K_{tc} of GSH (0.115/h) for fractional degradation (0.032/h) about 72% of K_{tc} will be related to a fractional digestion rate. In case of GSL only some 64% of the K_{ct} would be attributable to fractional digestion. The abovementioned calculations suggest that ^{13}C can be a tool to quantify the relations between digestion and fermentation along the gastro-intestinal tract.

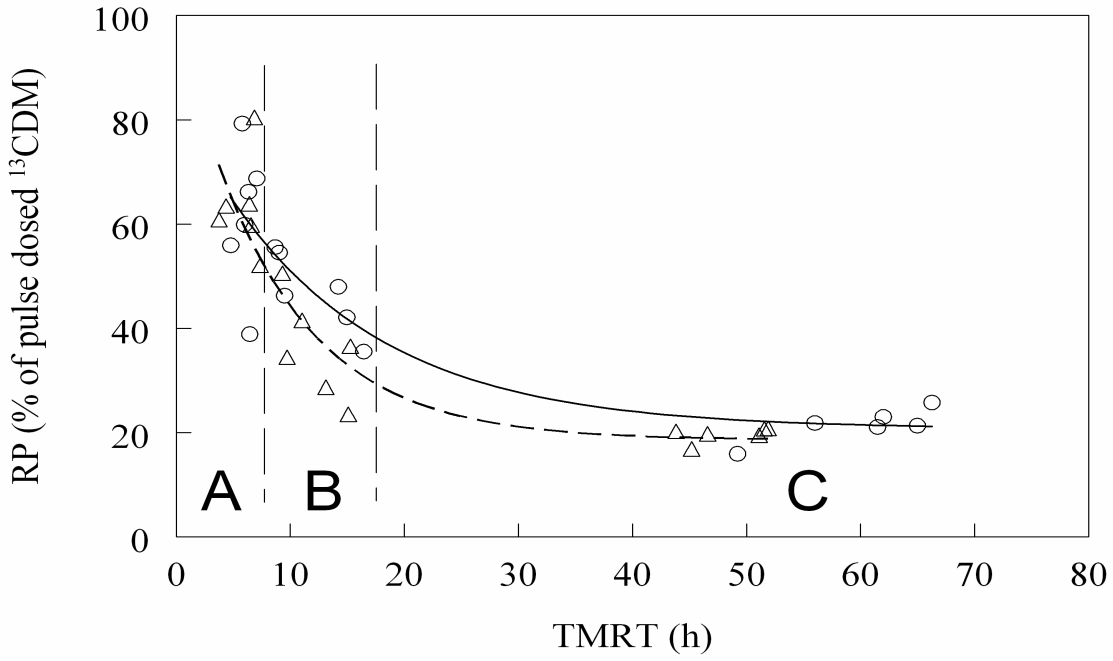

Figure 2. The faecal ^{13}CDM recoveries (RP, in %) upon pulse dosing in the ileum (A), abomasum (B) or rumen (C), in relation to the corresponding total mean residence times (TMRT), for GSL (———○———; $Y = 20.7+(100-16.3-20.7)\cdot\exp^{(-0.073\times TMRT)}$; $R^2 = 0.82$) and GSH (- - - - - - -Δ- - - - - - - ; $Y = 18.6+(100-0.0-18.6)\cdot\exp^{(-0.115\times TMRT)}$; $R^2 = 0.83$)

A point of consideration is that inherent to the choice of model a constant value for the fractional rate parameter is assumed, which is improbable. The fermentative processes in the hindgut differ from that in the rumen, whilst in the small intestine the emphasis will be on digestion with some fermentation in the ileum. Therefore, most likely each of these compartments will have its own specific rate constant. Besides, the pulse dosed silage is of inhomogeneous composition, which also implies the existence of more than 1 fractional rate constant. Therefore, exact interpretation of these calculations remains unclear, and would require more information.

Conclusions

Fractional passage rates between Co, Cr and ^{13}CDM upon ileal pulse dosing did not differ and were not affected by silage type. Upon abomasal pulse dosing differences in passage characteristics between markers became more pronounced, but not significant. Faecal excretion patterns of internal markers (^{13}CDM, ^{13}CNDR, ^{13}CNDS) following from rumen pulse doses, differed from those of the external markers. Between the internal markers, ^{13}CNDR had the lowest fractional passage rates from the slowest (K_1) and second slowest (K_2) compartment, had a more delayed transit time and longest total mean residence time. ^{13}C-labelled grass silage of high digestibility (GSH) tended to higher fractional passage rates compared to silage of low digestibility (GSL), and hence, to lower rumen and total tract residence times. The external markers did not show such differences between silage treatments. Moreover, it was demonstrated that the external marker Cr overestimates the fractional passage rates for both the DM and cell wall fractions. Therefore, we suggest that the use of ^{13}C as an internal marker enables to discriminate for feed component specific passage kinetics, and has a higher ability to differentiate between passage characteristics of the dietary treatments used in the current experiment, hence making ^{13}C superior to Cr. The recoveries (RP) of ^{13}C upon introduction in the ileum, abomasum rumen appeared to be non-linearly related to the mean residence times in the successive compartments. This suggests that ^{13}C can be a tool to quantify the relations between digestion and fermentation along the gastro-intestinal tract.

Acknowledgements

This work was co-financed by the Dutch Commodity Board of Feedstuffs, which is gratefully acknowledged. The authors kindly acknowledge the technical assistance of Mr. W. van Gestel, Mr. A. van den Dool, Mr. W. Nusselder, Mrs. I. van ‘t Anker, Mr. R. Wormgoor, Mr. W. van der Laan, Mr. L. Bijl, and analytical assistance of Mrs. M. van ‘t End, Mrs. S.C.W. Lammers-Wienhoven, Miss. J. M. Muylaert and Miss. G. Post. Contributions of R. Oomen, Mrs. J. Cerqueira-Lopes Pellikaan, Ms. S. Hemmer, Mr. S. Uiterwaal, and Mr. F. Jorna, as part of their undergraduate course are highly appreciated.

Literature Cited

AFRC. 1992. Nutritive requirements of ruminant animals: protein. AFRC Technical Committee on Responses to Nutrients. Report no. 9. Nutr. Abstr. Rev. (Series B). 62:787-835.

AFRC. 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. AFRC Technical Committee on Responses to Nutrients. Report no. 11. Nutr. Abstr. Rev. (Series B). 68:938-989.

Ankom Technology, 2003. Subject: NDF procedure by use of F57-type filter bags. http://www.ankom.com/09_procedures/procedures2.shmtl. Accessed June 20, 2003.

Baldwin, R.L. 1995. Modeling ruminant digestion and metabolism. Chapman and Hall, London.

Beauchemin, K. A. and J. G. Buchanan Smith. 1989. Evaluation of markers, sampling sites and models for estimating rates of passage of silage or hay in dairy cows. Anim. Feed Sci. Technol. 27(1-2):59-75.

Bibby, J., and H. Toutenburg. 1977. Prediction and improved estimation in linear models. John Wiley and Sons, London.

Bosch, M.W. 1991. Influence of stage of maturity of grass silages on digestion processes in dairy cows. Ph.D. Diss., Wageningen Agricultural University, The Netherlands.

Bosch, M.W., S.C.W. Lammers-Wienhoven, G.A. Bangma, H. Boer, and P.W.M. van Adrichem. 1992. Influence of stage of maturity of grass silages on digestion processes in dairy cows. 2. Rumen contents, passage rates, distribution of rumen and faecal particle and mastication activity. Livest. Prod. Sci. 32: 265-281.

Bruining, M. and M. W. Bosch. 1992. Ruminal passage rate as affected by CrNDF particle size. Anim. Feed Sci. Technol. 37(3-4):193-200.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, I. McMillan, and D. J. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. J. Dairy Sc. 73(8):2143-2156.

De Visser, H, H. Huisert, A. Klop, and R.S. Ketelaar. 1993. Autumn-cut grass silage as roughage component in dairy cow rations. 2. Rumen degradation, fermentation and kinetics. Neth. J. Agric. Sci., 41:

Dhanoa, M.S., R.C. Siddons, J. France, and D.L. Gale. 1985. A multicompartmental model to describe marker excretion patterns in ruminant faeces. Br. J. Nutr. 53:663-671.

Dijkstra, J., and J. France. 1996. A comparative evaluation of models of whole rumen function. Ann. Zootechnie. 45:175-192.

Dijkstra, J, J. France, and D.R. Davies. 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. J. Dairy Sci. 81: 3370-3384.

Egan, J. K., and P. T. Doyle. 1984. A comparison of particulate markers for the estimation of digesta flow from the abomasum of sheep offered chopped oaten hay. Aust. J. Agric. Res. 35:279-291.

Gasa, J., K. Holtenius, J. D. Sutton, M. S. Dhanoa, and D. J. Napper. 1991. Rumen fill and digesta kinetics in lactating Friesian cows given two levels of concentrates with two types of grass silage ad lib. Br. J. Nutr. 66(3):381-398.

Goelema, J.O., M.A.M. Spreeuwenberg, G. Hof, A.F.B. van der Poel, and S. Tamminga. 1998. Effect of pressure toasting on the rumen degradability and intestinal degradability of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Anim. Feed Sci. Technol. 76:35-50.

Huhtanen, P., and A.N. Hristov. 2001. Estimating passage kinetics using fibre-bound ^{15}N as an internal marker. Anim. Feed Sci. Technol. 94:29-41.

Huhtanen, P., and U. Kukkonen. 1995. Comparison of methods, markers, sampling sites and models for estimating digesta passage kinetics in cattle fed at two levels of intake. Anim. Feed Sci. Technol. 52:141-158.

Hume, I.D., R.L. Moir and M. Somers. 1970. Synthesis of microbial protein in the rumen. I. Influence of the level of N intake. Austral. J. Agric. Res. 21:283-296.

Kokkonen, T., M. Tuori, and L. Syrjälä-Qvist. 2000. Effect of silage dry matter content and rapeseed meal supplementation on dairy cows 2. Rumen fermentation and digesta passage rate. Anim. Feed Sci. Technol. 84:229-242.

Lund, P. 2002. The effect of forage type on passage kinetics and digestibility of fibre in dairy cows. Ph.D. Diss., The Royal Veterinary and Agricultural University Copenhagen, Denmark.

Mambrini, M., and J.L. Peyraud. 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculations based on faecal collection. Reprod. Nutr. Dev. 37:427-442.

O'Connor, J.D., P.H. Robinson, C.J. Sniffen, and M.S. Allen. 1984. A gastro-intestinal tract simulation model of digesta flow in ruminants. Pages 102-121 in Techniques in particle size analysis of feed and digesta in ruminants. Ed. P.M. Kennedy. Can. Soc. Anim. Sci. occasional publication 1, Edmonton, Alberta, Canada.

Offer, N.W. and J. Dixon. 2000. Factors affecting outflow rate from the reticulo-rumen. Nutr. Abstr. Rev. (Ser. B), Livestock Feeds and Feeding. 70(11):833-844.

Owens, F.N., and A.L. Goetsch .1986. Digesta passage and microbial protein synthesis. In Control of Digestion and Metabolism in Ruminants, pp. 196-223 [LP Milligan, WL Grovum and A Dobson, editors] Prentice-Hall, Englewood Cliffs.

Pellikaan, W.F., H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven, and S. Tamminga. 2004[a]. ^{13}C-enrichment of cell wall fractions of rye grass (*Lolium perenne* L.) and implications for its use as digesta marker. Submitted to J. Anim. Sci.

Pellikaan, W.F., J. Dijkstra, S. Tamminga, H. Boer, S.C.W. Lammers-Wienhoven, W.J.H. Van Gestel and G. Hof. 2004[b]. Passage of ^{13}C-labelled grass silage through the gastro-intestinal tract of dairy cows at two levels of feed intake. Submitted J. Anim. Sci.

Pellikaan, W.F., J. Dijkstra, G. Hof, B.M. Tas, H. Boer, and S. Tamminga. 2004[c]. Passage of ^{13}C-labelled grass through the gastro-intestinal tract of dairy cows. Submitted to Br. J. Nutr.

Pond, K.R., W.C. Ellis, J.H. Matis, H.M. Ferreiro, and J.D. Sutton. 1988. Compartment models for estimating attributes of digesta flow in cattle. Brit. J. Nutr. 60:571-595.

Rinne, M., P. Huhtanen, and S. Jaakkola. 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol. 67:19-35.

Robinson, P.H., J.G. Fadel, and S. Tamminga. 1986. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Anim. Feed Sci. Technol. 15:249-271.

Roffler, R.E., C.G. Schwab and L.D. Satter. 1974. Influence of urea intake on ruminal ammonia concentration. J. Dairy Sci. 57:631-633.

Satter, L.D. and L.L. Slyter 1974. Effect of rumen ammonia concentration on rumen microbial production *in vitro*. Brit. J. Nutr. 34:199-208.

Tamminga, S., P.H. Robinson, M. Voigt, and H. Boer. 1989. Rumen ingesta kinetics of cell wall components in dairy cows. Anim. Feed Sci. Technol. 25:89-98.

Tamminga, S., W.M. Van Straalen, A.P.J. Subnel, R.G.M. Meijer, A. Steg, C.J.G. Wever, and M.C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40:139-155

Tamminga, S., and A.M. Van Vuuren. 1988. Formation and utilization of end products of lignocellulose degradation in ruminants. Anim. Feed Sci. Technol. 21:141-159.

Thiago, L. R. L., M. Gill, and M. S. Dhanoa. 1992. Studies of method of conserving grass herbage and frequency of feeding in cattle. 1. Voluntary feed intake, digestion and rate of passage. Brit. J. Nutr. 67: 305-318.

Tóthi, R. 2003. Processed grains as a supplement to lactating dairy cows. Ph.D. Diss., Wageningen University, The Netherlands.

Udén, P., P.E. Colucci, and P.J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J. Sci. Food Agric. 31:625-632.

Valk, H., I.E. Kappers, and S. Tamminga. 1996. *In sacco* degradation characteristics of organic matter, neutral detergent fibre and crude protein of fresh grass fertilized with different amounts of nitrogen. Anim. Feed Sci. Technol. 63:63-87.

Van Soest, P.J. 1973. Collaborative study of acid-detergent fiber and lignin. J. Assoc. Offic. Anal. Chem. 56:781-784.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vérité, R., B. Michalet-Doreau, P. Chapoutot, J.L. Peyraud and C. Poncet. 1987. Révision du systeme des protéines digestibles dans l'intestin (PDI). Bulletin Technique C.R.Z.V. Theix, INRA. 70:19-34.

Yang, W.Z. K.A. Beauchemin, and L.M. Rode. 2001. Barley processing, forage:concentrate, and forage length effects on chewing and digesta passage in lactating cows. J. Dairy Sci. 84:2709-2720.

Chapter 6

Use of Mathematical Models and Compartmentalisation of the Dairy Cow's GIT

W.F. Pellikaan, J. Dijkstra, and S. Tamminga

Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands

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W.F. Pellikaan, J. Dijkstra, and S. Tamminga

Abstract

Marker excretion patterns from four separate animal trials were combined, resulting in a dataset consisting of 338 faecally and ileally determined marker excretion curves. This dataset was used to compare parameter estimates obtained from curvefitting using a deterministic multi-compartmental model (MC-model) with those from several two-compartmental stochastic models with increasing order of gamma-distributions (GnG1; n = 1 to 5). The determination coefficient (R^2) and a regression coefficient (α), which describes the relation between the observed and predicted marker concentrations, were used to determine the 'goodness of fit'. The results were classified into three categories: Good fits (GF, $R^2 \geq 0.9$ and $\alpha \geq 0.9$); False fits (FF, $R^2 < 0.9$ and $\alpha < 0.9$); False positive fits (FP, $R^2 \geq 0.9$ and $\alpha < 0.9$). Data suggested that models with higher order gamma-distributions (n > 2) and the MC-model were about equally succesfull in fitting excretion patterns. However, the fractional rate constants describing the outflow from the slowest compartment (K_1) were consistently higher for these GnG1-models compared to the MC-model. Comparison between GnG1-models showed that an increase in the order of gamma-distributions resulted in higher values for K_1, but also increased the variation.

The MC-model gives information on the residence times for the slowest compartment (CMRT1; rumen), the second slowest compartment (CMRT2; caecum/ large intestine) and the transit time (TT; lognitudinal displacement in the tubular sections). The use of multiple cannulated animals made it possible to test whether the parameter estimates obtained from faecal excretion patterns indeed relate to the above mentioned physical compartments. CMRT1-values estimated from ileal and faecal excretion following from pulse doses in the rumen tended towards a linear relation, but the correlation was low due to large variation. The faecally determined CMRT2 obtained after rumen pulse dosing, showed considerable more variation in residence times between different markers than the directly measured residence time in the caecum/ large intestine compartmentafter pulse dosing into the ileum. With regard to the TT it proved to be unlikely that this item is solely related to the longitudinal displacement in the tubular section of the GITs. Moreover, date suggested large differences between markers regarding the time spend in the small intestine compartment.

Finally the dataset was used to study the relation between some animal and feed characteristics, and the different compartmental residence times. The results suggested that the residence times (CMRT1, CMRT2, TT) of the particle phase markers can be reasonably well predicted from a combination of animal and feed characteristics. Comparing our observed fractional rate constants with estimates obtained from equations presented by Van Straalen (1995), Pitt *et al.* (1992), Sniffen *et al.* (1992) and Owens and Goetsch (1986) showed that the observed variation within and between markers was not reflected in their equations. This can be partly explained by the type and number of regressors included in the various equations. On the other hand, our number of data points was limited, our experimental conditions were specific and the biological relevance of the determinants were in some cases difficult to interpret. Nevertheless, our data suggest that these multiple regression equations based on animal and feed characteristics form interesting tools for feed evaluation programs to adapt rate constants to on-farm situations.

Keywords: stochastic and deterministic nonlinear regression models, compartments, markers, fractional passage rates, dairy cows

Introduction

Approaches in Mathematical Modeling

Over the years, a range of mathematical models that describe marker excretion kinetics following single pulse dose administration has been presented. Early marker kinetics studies of Balch (1950) clearly showed how rate of passage affects the intake and digestibility. Campling and Freer (1962) made first attempts to delineate the effect of specific gravity and particle size in relation to their mean retention times in the gastro-intestinal tract (GI-tract) of cows. First serious attempts to model marker kinetics were done by Blaxter *et al.* (1956). In their model, they included two exponential terms to describe the two mixing compartments and a time delay to represent the tubular sections. They observed that one of the rate constants (in their model K_1) was very sensitive to changes in level of feed intake and feed particle size. Although their work did not provide direct evidence, they concluded that the higher and more variable rate constant (K_1) and the time delay (TT) both represented events taking place in the rumen, whilst the slower rate constant (K_2) was associated with the post-rumen compartments. Initially however, they schematically assigned the TT to the time delay caused by the segment after the duodenum, and the K_1 and K_2 to respectively the reticulo-rumen-omasum and the abomasum. Grovum and Williams (1973) proposed a double exponential model similar to the Blaxter model. They specifically assigned the more gradual declining part of the excretion curve to events in the rumen (K_1), the shorter and rapidly increasing part of the curve to marker passage through the caecum and proximal colon (K_2) and the time delay (transit time, TT) to digesta passage through the intestines. With this, they challenged the model interpretations of Blaxter *et al.* (1956) and stated that Blaxter's rate constant K_1 was probably more representative for the caecum-proximal colon part whilst the rate constant K_2 more likely resembled the rumen. Based on the same experimental information and the same model, Grovum and Philips (1973) concluded that when model predictions do not meet the actual marker excretion pattern this indicates that the two-compartmental model is not representative for the marker passage, and hence, leaves room to develop new models. From the early '70's onwards a number of models have been proposed, which can be assigned to two approaches in mathematical modelling; a deterministic and a stochastic approach. Both approaches aim to describe marker excretion patterns through mathematical models that generate fractional rate constants, which are supposedly representative for corresponding compartments of the GI-tract determined by those models.

Stochastic modeling. Stochastic models include probability distributions that allow predicting the expected parameter values (e.g. fractional rate constants) and their accompanying variances. Based on this approach, Matis (1972) included a so-called gamma time-dependency factor in the first compartment (i.e., the rumen) of Blaxter's *et al.* (1956) two-compartmental model. However, with regard to the conclusions of Grovum and Williams the lifetimes would actually be associated with processes in the caecum-proximal colon. The concept behind these stochastic models is that with prolonged residence times particles have a

progressively higher probability to escape from a compartment. To account for this process, a so-called Erlang distribution or integer gamma has been included in the model (Matis *et al.*, 1989; Matis, 1972), where the rate function (lambda) describing this distribution of the residence times increases with time from zero to a constant rate asymptote. In the subsequent years this concept has been further worked out and evaluated (Lund, 2002; Wylie *et al.*, 2000; Moore et al, 1992; Poore *et al.*, 1991; Matis *et al.*, 1989; Pond *et al.*, 1988). Pond *et al.* (1988) give a full reference to the use of two-compartment age-dependent, age-independent (GnG1) models and specifically mention that these models do not specify the order or relative size of the two sequential compartments. However, experience shows that the age-dependency is consistently related to the compartment with the higher rate constant. Furthermore, they assume that the ingested feed particles first enter this latter compartment before entering the age-independent compartment (with the lower rate constants), i.e., suggesting that digesta passage is solely determined by different processes in the rumen. By introducing a time delay into their equations (see also Table 1), a correction is made for the segments of the GI-tract where the flow of digesta occurs through non-mixing displacement. Non-mixing displacement or prop-flow is in general associated with the more tubular sections of the GI-tract (O'Connor *et al.*, 1984). By introducing different markers at different sites into the GI-tract of dairy cows in combination with duodenal and faecal sampling, Wylie *et al.* (2000) showed that the influence of post-ruminal mixing pools on faecally determined rumen rate parameters (λ_2, K_1; Table 1) was negligible. The general approach in passage studies based on stochastic models is that the ascending part of the faecal excretion curve is explained by events in the pre-duodenal compartments (Wylie *et al.*, 2000; Huhtanen and Kukkonen, 1995; Pond *et al.*, 1988).

Deterministic modeling. Deterministic models generate definite predictions of the expected parameter values without accompanying variances (France and Thornley, 1984; Matis *et al.*, 1989). Dhanoa *et al.* (1985) developed a deterministic multi-compartmental model, in which the GI-tract is represented by an unspecified number of sequential compartments, where the

Table 1. Different models used to describe the marker excretion patterns.

Model	Notation	MRT
G1G1	$A \cdot K_1 \cdot [e^{-K_1 \cdot T} - e^{-K_2 \cdot T}] / (K_2 - K_1)$	$1/K_1 + 1/K_2$
G2G1	$A \cdot \{\delta^2 \cdot e^{-K_1 \cdot T} - [e^{-\lambda_2 \cdot T} \cdot (\delta^2 + (\delta \cdot \lambda_2 \cdot T))]\}$	$1/K_1 + 2/\lambda_2$
G3G1	$A \cdot \{\delta^3 \cdot e^{-K_1 \cdot T} - [e^{-\lambda_2 \cdot T} \cdot \{\delta^3 + (\delta^2 \cdot \lambda_2 \cdot T) + (\delta \cdot \lambda_2^2 \cdot T^2 / 2)\}]\}$	$1/K_1 + 3/\lambda_2$
G4G1	$A \cdot \{\delta^4 \cdot e^{-K_1 \cdot T} - [e^{-\lambda_2 \cdot T} \cdot \{\delta^4 + (\delta^3 \cdot \lambda_2 \cdot T) + (\delta^2 \cdot \lambda_2^2 \cdot T^2 / 2) + (\delta \cdot \lambda_2^3 \cdot T^3 / 6)\}]\}$	$1/K_1 + 4/\lambda_2$
G5G1	$A \cdot \{\delta^5 \cdot e^{-K_1 \cdot T} - [e^{-\lambda_2 \cdot T} \cdot \{\delta^5 + (\delta^4 \cdot \lambda_2 \cdot T) + (\delta^3 \cdot \lambda_2^2 \cdot T^2 / 2) + (\delta^2 \cdot \lambda_2^3 \cdot T^3 / 6) + (\delta \cdot \lambda_2^4 \cdot T^4 / 24)\}]\}$	$1/K_1 + 5/\lambda_2$
MC	$A \cdot e^{-(K_1 \cdot t)} \cdot \exp[-(N-2) \cdot e^{-(K_2 - K_1) \cdot t}]$	$1/K_1 + 1/K_2$

A = Initial marker concentration in the compartment where marker was introduced; K_1 = Fractional rate constant for the slowest compartment; K_2 = Fractional rate constant for the second slowest compartment; λ_2 = Fractional rate parameter for the gamma-distributed residence times in the second (slowest) compartment; $\delta = \lambda_2/(\lambda_2 - K_1)$; t = time after pulse dose; T = (t – TT), where TT represents a time delay.

two compartments with the lowest fractional rate constants predominantly determine the pattern of marker excretion. The concept here is that processes within each successive compartment follow first-order kinetics and are irreversible. In contrast to the stochastic models the ascending part of the excretion curve is here being interpreted as a representation of events in the caecum/ proximal-colon compartment. Therefore, comparison of individual rate constants that are related to specific compartments or events in the GI-tract is difficult, and strongly depends on the choice of model. Therefore, information on the total mean retention time has greater statistical power and will be less affected by model configurations (Matis *et al.* 1989). However, than the information on digesta passage through specific physical compartments in the GI-tract remains obscured.
Mathematical explanations and philosophical considerations of both approaches can be found amongst others in France *et al.* (1989) and Matis *et al.* (1989). Several comparative studies have shown that the use of different models and approaches give a comparable variability in values for rate constants and parameter estimates (e.g. Bernard *et al.*, 1998; Mambrini and Peyraud, 1994). In conclusion, Poppi *et al.* (1999) appropriately state that "the choice of approach is as much a philosophical outlook on parameter estimations as it is on the availability of packages and procedures to use".

Compartmentalising the ruminant gastro-intestinal tract. Already from early to mid 19th century work has been carried out to relate feed quality parameters to the requirements of farm animals. Classic works of Henneberg and Stohmann (1860) and Wolf (1861) show that already at that time considerable efforts were made to acquire knowledge on animal nutrition. However, with regard to the ruminant animals the process of digestion in these early works was approached from a 'black box' principle as specific knowledge on, for instance, rumen functioning was largely lacking. In 'Principles of Animal Nutrition', Armsby (1914) proposes to adopt the term '*apparent*' in combination with '*digestibility*', to correct the '*real digestibility*' for the loss through digestive fluids, intestinal mucus, epithelium, etc. He also mentions the general awareness of the complicated digestive apparatus of herbivorous species, and that a variety of processes (e.g., fermentation) obscure the 'simple solution of nutrients by digestive fluids' as happens in monogastrics. Although the importance of the fermentative processes in the digestive tract of herbivores is acknowledged, its extent and contribution to the animal's nutrient supply could not yet be valued. The technical and methodological developments, the accompanying increase of knowledge and the seemingly concomitant increase in questions, directed nutrition research more and more into a reductionistic direction up to the present situation where nutrition and nutrient dynamics are being studied at various levels (cell, organ, animal). The focus of this thesis, digesta passage through the gastro-intestinal tract (GI-tract), represents just one level in the multi-factorial and complex topic of nutrition physiology. During the past 50 years, numerous techniques have been developed to study passage behaviour of digesta through the GI-tract of ruminants in order to refine the knowledge of nutrient utilisation in the different parts (compartments) of the tract. The use of different types of markers (e.g. external vs. internal), the techniques of

application (e.g. pulse vs. continuous, and/ or spot sampling vs. total evacuation), the use of multiple-cannulated animals, and the development of mathematical models describing the marker excretion patterns, allowed to 'compartmentalise' the GI-tract of the ruminant animal. A major issue in passage studies is to compartmentalise the animal's intestinal tract based on faecal excretion patterns. Increasing restrictions caused by legislative regulation on the use of experimental animals (De Greeve *et al.*, 1993), but also the discussion on the representability of using intestinally cannulated animals (Harmon and Richards, 1997; Faichney, 1975; MacRae, 1975) make it desirable to derive this information from faecal excretion patterns using intact animals.

Comparison of Models

In the preceding chapters marker excretion patterns have been analysed using the multi-compartmental model (MC-model) as described by Dhanoa *et al.* (1985). The choice to use this deterministic model was twofold. Firstly, preliminary work (Roordink, 1999; unpublished data) on curve fitting indicated the robustness of the MC-model compared to other available models. Secondly, in The Netherlands an inventory study has been initiated to develop a new feed evaluation system, with a more dynamic, mechanistic, and deterministic nature (Tamminga *et al.*, 2000). The deterministic approach of the MC-model seeks to make definite predictions of retention times in the successive compartments. Therefore, the concept of this model is in line with the argumentations of the models underlying the novel feed evaluation system. As stated earlier, the different models will generate different fractional passage rate constants for the concomitant compartments. The combined information on the external and internal marker excretion patterns of the successive animal experiments resulted in a considerable dataset that allows to assess the differences between parameter estimates of different models. The gamma-distributed stochastic two-compartmental models as presented by Pond *et al.* (1988) were compared with the MC-model described by Dhanoa *et al.* (1985). The mathematical notations of the two-compartmental models with various orders of gamma-distributions (G1G1 to G5G1) and the MC-model shown in Table 1, describe the change in marker concentration in faecal and ileal chyme samples. The total dataset consisted of 338 marker excretion patterns that were subsequently fitted to six different models, resulting in a total of 2028 curve fits. A full description on how the excretion patterns were obtained is given in the preceding chapters (3, 4 and 5). The model notations (Table 1) were programmed in SAS using the NLIN procedure (SAS Inst. Inc., Cary, NC, 1996). In addition to the model parameters (fractional passage rate constants), the model SSE, MSE, the determination coefficients (R^2), the mean prediction error (MPE; Bibby and Toutenburg, 1977) and the regression coefficient (α) describing the relation between the observed and the predicted values were determined. A summary of the curve fit results is given in Table 2. Curve fitting resulted in 37 of the 2028 occasions in a non-convergence; for G1G1 (1), G4G1 (4), G5G1 (4)

and MC (28), suggesting poorer results with the MC-model. However, further examination proved that in 27 cases the non-convergence was false (for MC 24), hence, in only 10 cases curve fit solutions truly gave a non-convergence. On eight occasions extreme outlying values were obtained for the values for K_1 (> 1000%/h) K_2 (> 450%/h) and/ or N (> 10^6). These observations were excluded from futher calculations, resulting in a dataset of 2010 curve fits (Table 2). The non-convergence and outlying values occured mainly for excretion curves obtained after ileal and abomasal pulse dose administrations and was associated with a low number of measurement points before peak concentration was reached.

To determine the 'goodness of fit' of the different models, a combination of the R^2 (squared correlation coefficient) and the α was used to group the curve fits into three categories: Good fits (GF, $R^2 \geq 0.9$ and $\alpha \geq 0.9$); False fits (FF, $R^2 < 0.9$ and $\alpha < 0.9$); False positive fits (FP, $R^2 \geq 0.9$ and $\alpha < 0.9$). The combined results of the FF and FP are summarised in Table 2. From the total number of 2010 curves 96 were false positive whilst in

Table 2. The combined numbers of false fits (FF) and false positive fits (FP) after fitting the total number of faecal (F) and ileal (I) excretion curves per pulse dose site to different models (stochastic, GnG1; deterministic, MC), and after evaluating the total number of curve fits per marker (Cr, Co, ^{13}CDM, ^{13}CNDR, ^{13}CNDS).

Item	Over all		Ileum	Abomasum	Reticulo-rumen	
	No. of curves	FF+FP[1]	(F)	(F)	(I)	(F)
Total curves	2010		420	429	579	582
Models		*FF + FP*[1]				
G1G1	338	280	68	71	47	94
G2G1	338	188	54	70	12	52
G3G1	336	106	32	64	4	6
G4G1	335	100	31	59	5	5
G5G1	333	92	34	47	8	3
MC	330	50	29	10	7	4
Sum FF+FP[1]		816	248	321	83	164
Markers		*FF + FP*[1]				
Cr	476	204	68	86	14	36
Co	476	220	79	102	0	39
^{13}CDM	465	197	71	92	13	21
^{13}CNDR	296	115	14	19	42	40
^{13}CNDS	297	80	16	22	14	28
Sum FF+FP[1]		816	248	321	83	164

[1] The summed number of the false fits and false positive fits, determined by the following curve fit criteria: FP = False positive fit, $R^2 \geq 0.9$ and $\alpha < 0.9$; FF = False fit, $R^2 < 0.9$ and $\alpha < 0.9$; GF = Good fit, $R^2 \geq 0.9$ and $\alpha \geq 0.9$; where R^2 equals the model determination coefficient and α equals the regression coefficient of the observed on the predicted values.

720 cases the fits were false, resulting in a total of 816 erroneous fits (FF + FP, about 41%). Visual comparison of the fitted curves through the observed marker concentration points in combination with the associated α-values (observed vs. predicted) indicated that especially the G1G1 and G2G1 model gave very poor fits, respectively 280 and 188 FF + FP out of 676 curves (Table 2). Only one case gave a non-convergence and output statistics did not directly indicate to problems in giving erroneous solutions. This could mean that these model solutions were obtained from so-called local minima. However, the starting values to initiate the iterative process were obtained from a grid search in the full parameter space to prevent solutions from local minima (See former chapters for criteria). Therefore, these results nicely illustrate the importance of visual examination of curve fit results. Within the 330 curves fitted by the MC-model 11 curves were FP and 39 were FF. Only the G5G1 model gave lower counts for FP (6 out of 333 curves), but FF counted 86, resulting in an over-all poorer fit. The G1G1 and G2G1 models gave the poorest fits, whilst the models with higher gamma-distributions and the MC-model appeared to be similarly successful in fitting excretion patterns, especially upon pulse dosing into the rumen. In general, the excretion patterns after abomasal pulse dosing occurred to be most difficult to fit by the models followed by the excretion patterns obtained after ileal pulse doses. The MC-model gave the lowest number of FF + FP, and hence, seemed to be the most robust model.

The total number of curve fits of ^{13}C in the cell wall (NDR) and non-cell wall (NDS) fractions is lower than the other markers. Only in the first two experiments (Chapter 3) these excretion patterns were also established after pulse dosing into the ileum and abomasum. In the other experiments only ileal and faecal excretion patterns of ^{13}CNDR and ^{13}CNDS were obtained following from ruminal pulse doses. When scaling the number of FF and FP of ^{13}CNDR (115) and ^{13}CNDS (80) to their total number of curves (respectively 296 and 297) the relative percentage of erroneous fits (FF + FP) ranges between 27% and 39%. In case of scaling Cr, Co and ^{13}CDM to their total number of curves, the relative percentages are slightly higher (42% to 46%). From these results it may be concluded that the excretion patterns of the novel marker (^{13}C) can be fitted as accurately as those of the external markers. Furthermore, these results show that the models with higher gamma-distributions ($n \geq 3$) and the MC-model give comparable accuracies in curve fitting.

In addition to the R^2, the differences in curve fitting between models can be evaluated by the model mean square error (MSE, Huhtanen and Hristov, 2001; Pond *et al.*, 1988), the root MSE (Huhtanen *et al.*, 1995; Motulsky and Ransnas, 1987), the residual sum of squares (SSE, Amici *et al.*, 1997; Moore *et al.*, 1992; Quiroz *et al.*, 1988) and/ or the mean squared prediction error (MSPE, Dhanoa *et al.*, 1989; Aichison *et al.*, 1986; Bibby and Toutenburg, 1977). However, before evaluating the goodness of fit based on these statistical parameters, predicted values of individual model fits should be superimposed on the actual data points. Through this exercise, potential problems like model solutions at local minima in the sum of squares surface can be recognized (Motulsky and Ransnas, 1987). The root MSE describes the average deviation of the curve fit from the actual points (Motulsky and Ransnas, 1987). The

SSE accounts more for deviations around the peak of the curve, hence, model selection based on this parameter may give a preference for models that give good estimations around the peak whilst estimations at both ends of the curve are poorer (Moore *et al.* 1992). The MSE allows selecting models that give more accurate fits at the end of the curve (Udén *et al.*, 1982). When using the R^2, SSE, MSE and root MSE to compare models, one has to bear in mind that these statistics are affected by the difference in model parameters estimated by the models. On one hand, an increase in the number of parameters in a model in general results in better fits, however, the loss of degrees of freedom will negatively influence the residual variance and accuracy of a model. Motulsky and Ransnas (1987, p. 371) presented an adjusted *F*-test that corrects for this, however, the limitation here is that the test can compare between only two models. Another problem arising is that large quantitative differences in excretion concentrations between the different markers (e.g., Cr versus ^{13}C) will result in large differences in the SSE and the MSE, which makes a direct comparison between markers inappropriate. A method that circumvents these problems is the MSPE. The MSPE can be decomposed into (i) errors in central tendency, (ii) errors due to deviation of the regression slope from one and (iii) errors due to random disturbance (Bibby and Toutenburg 1977), and hence, allows to distinguish in what way the accuracy of the curve fit is dominantly affected. By scaling the root of the MSPE to the observed mean (MPE) this factor can be easily used to compare between model fits. Furthermore, the MSPE (or MPE) solely reflects the error related to the model curve fit irrespective of the number of parameters fitted by that model, which makes it a robust method to compare models. To test for differences between models and markers the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, 1996) according to the following model.

$$Y=\mu+G_i+M_j+(G\times M)_{ij}+A_k\{E+D+P\}_{lmn}+S_o\{T\}_p+(G\times A)_{ik}\{E+D+P\}_{lmn}+(G\times S)_{io}\{T\}_p+(G\times M\times S)_{ijo}\{T\}_p+e_{ijklmnop} \quad [1]$$

where, Y = dependent variable, μ = intercept, G_i = gamma, M_j = marker, A_k = animal, E_l = experiment, D_m = diet, P_n = period, S_o = sample, T_p = pulse dose treatment, interaction terms are included within parenthesis and nested effects within brackets. Some results for the main effects model (G) and marker (M) are summarised in Table 3. The mean values presented here are based on the 2010 curve fits (Table 2), meaning that the mean fractional passage rate constants are based on marker excretion patterns that follow from the various pulse dose sites. This explains the rather high mean values for fractional passage rates decribing the flow from the slowest (K_1) and second slowest (K_2) compartment. Regression of the predicted on the observed values (α) and MPE showed that the MC-model was superior to the gamma-distributed models. With an α-value of 0.60 and a MPE of 63.84% the G1G1 model gave the poorest fitting results. Increasing the order of gamma-distribution considerably improved the goodness of fit, which agrees with the general observations in literature (Lund, 2002; Hristov and Huhtanen, 2001; Amici *et al.*, 1997; Pond *et al.*, 1988). The K_1 gave significantly higher values for the GnG1-models (where $n > 2$) compared to the MC-model, whilst rate constants

Table 3. The least square means of the regression coefficient (α), the mean prediction error (MPE) and the fractional passage rates for the slowest compartment (K_1) and the second slowest compartment (K_2) for different models (GnG1 and MC) and markers (Cr, Co, ^{13}CDM, ^{13}CNDR, ^{13}CNDS).[1, 2]

Item	α	MPE	Fractional passage rates	
		(%)	K_1 (/h)	K_2 (/h)
Estimates for different models[3]				
G1G1	0.601^{a} (0.009)	63.84^{a} (1.45)	0.151^{a} (0.018)	0.439^{a} (0.046)
G2G1	0.758^{b} (0.009)	44.05^{b} (1.45)	0.314^{b} (0.018)	0.527^{a} (0.046)
G3G1	0.867^{c} (0.009)	35.60^{c} (1.46)	0.400^{c} (0.018)	0.764^{b} (0.046)
G4G1	0.881^{cd} (0.009)	31.66^{c} (1.46)	0.467^{c} (0.018)	0.931^{bc} (0.046)
G5G1	0.855^{c} (0.010)	34.55^{c} (1.52)	0.429^{c} (0.019)	1.079^{dc} (0.046)
MC	0.914^{d} (0.010)	24.81^{d} (1.55)	0.264^{b} (0.019)	1.170^{d} (0.049)
Estimates for different markers[3]				
Cr	0.817^{a} (0.006)	39.06^{b} (0.88)	0.359 (0.011)	0.679^{a} (0.028)
Co	0.766^{b} (0.006)	50.86^{a} (0.91)	0.351 (0.011)	0.787^{b} (0.028)
^{13}CDM	0.815^{a} (0.006)	39.67^{b} (0.89)	0.346 (0.011)	0.771^{ab} (0.028)
^{13}CNDR	0.827^{a} (0.010)	32.56^{c} (1.57)	0.329 (0.020)	0.721^{ab} (0.049)
^{13}CNDS	0.838^{a} (0.010)	33.28^{c} (1.59)	0.303 (0.020)	1.134^{c} (0.050)

[1] Mean values are based on dataset presented in Table 2 (2010 curves).
[2] α = regression coefficient derived by regressing the model predictions on the observed values; MPE = root MSPE scaled to the mean observed value (%); overall, regression and random scaled to the MPE (%).
[3] Different superscripts indicate significant differences between items in columns; Values between parenthesis show the SEM;

from the second slowest compartment (K_2) showed the opposite. Savoie *et al.* (2001) compared amongst others the G2G1-model (Pond *et al.*, 1988) and the MC-model (Dhanoa *et al.*, 1985). They illustrated that the G2G1-model has the potential to overestimate the rate constant associated with the declining part of the excretion curve. Pond *et al.* (1988) demonstrated the relation between a progressive increase in the order of gamma-distribution and an increased time delay (transit time) and slope of the declining line part (fractional passage rate constant from the rumen).

Figures 1 and 2 present the relation between K_1 as estimated by the stochastic models and the deterministic MC-model per site of pulse dose and sample collection. Figure 1 shows the over all relation between the five different orders of gamma-distributions and the MC-model. It clearly illustrates that the order of gamma-distribution has a considerable effect on the estimation of K_1, and that a higher order of gamma-distribution tends to increase the variation, i.e. increase the range of K_1. For both the stochastic models and the MC-model differences between particle phase markers become more evident after pulse dosing into the rumen, resulting in a more clustered pattern (Figure 2). Moreover, these clusters show considerable differences in the relationship between the different stochastic models and the MC-model. In conclusion it can be stated that a progressive increase in gamma-distribution results in higher values for the K_1 and an increase in variation. Fractional rate constants derived from ileal.

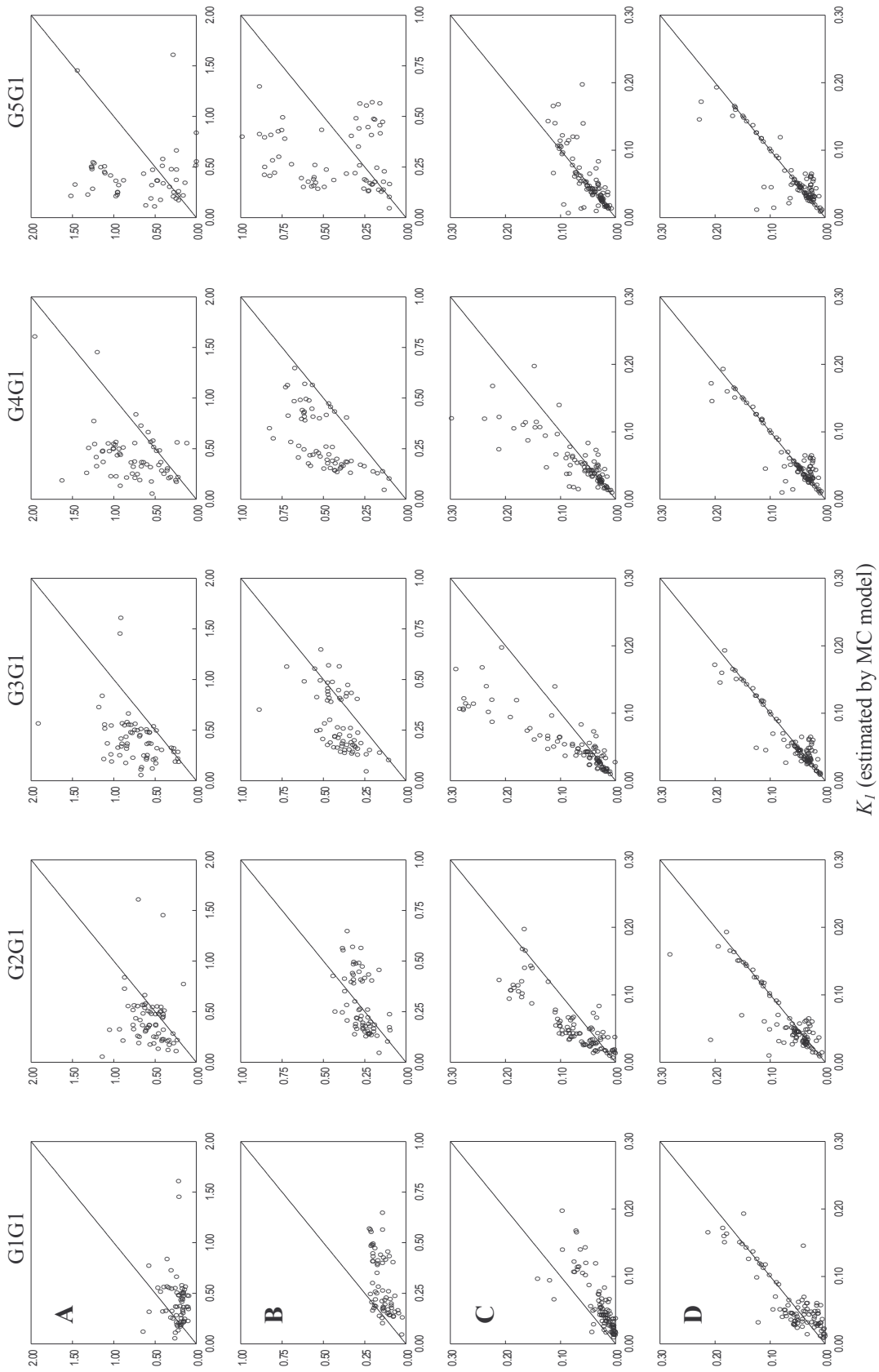

Figure 1. Relation between rate constants (K_1) as fitted by gamma-n models (G1G1 to G5G1) and the MC model from faecal excretion patterns after ileal (A), abomasal (B) and ruminal (C) pulse dose and from ileal excretion after ruminal pulse dose (D).

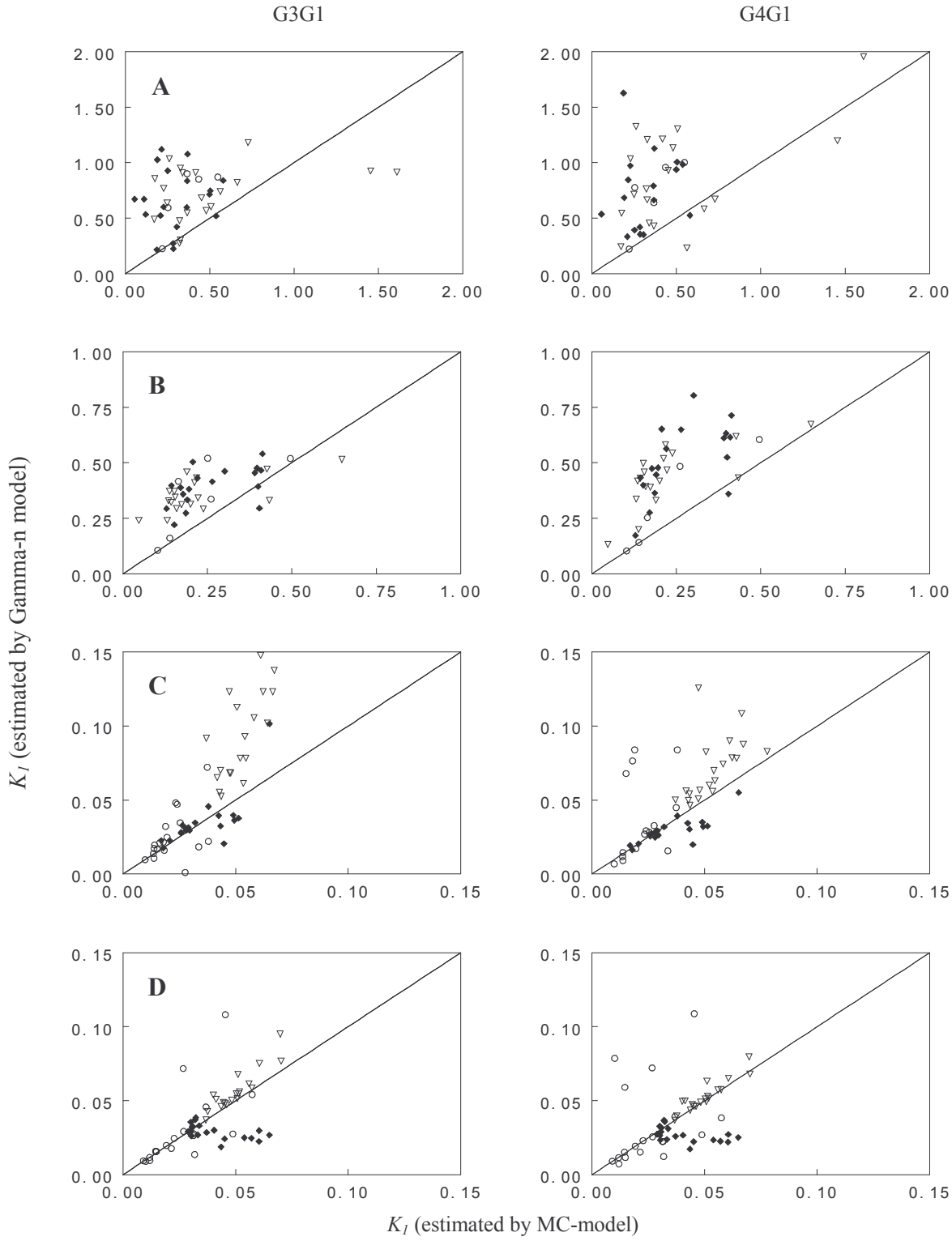

Figure 2. Relation between rate constants (K_1) as fitted by gamma-n models (G3G1 and G4G1) and the MC-model from faecal excretion patterns after ileal (A), abomasal (B) and ruminal (C) pulse dose, and from ileal excretion patterns following from ruminal pulse doses (D). Different symbols represent the fractional rate constants for chromium (∇), ^{13}C in the DM (♦) and ^{13}C in the NDR (o).

excretion patterns seem to be less sensitive to an increase in the order of gamma-distribution and hence, has a minor effect on the relation between stochastically and deterministically estimated K_1-values. Furthermore, two-compartmental stochastic models with higher orders of gamma-distribution ($2 < n < 6$) and the multi-compartmental deterministic model seem equally successful in fitting excretion patterns. However, rate constants describing the declining part of the excretion pattern and fitted by the stochastic models are consistently higher than those from the deterministic model. The novel marker ^{13}C (in DM, NDR and NDS) proved to be fitted as adequately as the traditionally used external markers Cr-NDF and Co-EDTA.

Compartmentalisation of the Gastro-Intestinal Tract in Ruminants

Mambrini and Peyraud (1997) used a simple graphic method to partition duodenal and faecal marker excretion curves into three sections (transit, ascending, descending) and derive the residence times (MRT's) related to the latter two sections from the natural logarithm. Subsequently, they tried to elucidate the faecally determined TT and MRT's by those obtained from duodenal excretions. They concluded that the faecally determined TT is mainly associated with the digesta transit through the tubular segments after the duodenum, but also part may be related to the time required for mixing of marker in the rumen or omasum. The MRT related to the ascending part of the faecal excretion curve, on the one hand reflects the time to reduce the size of forage particles, on the other that of the time used for compartmental mixing in the post-duodenal segments. However, no conclusive explanations of the MRT were given due to the possible presence of other unidentified compartments or processes.

In the animal trials described in the preceding chapters, rumen fistulated and ileum cannulated animals were used. An advantage compared to prior studies where abomasal and/or duodenal cannulated animals were used, is that such modified animals allow to specifically study digesta passage through the caecum-large intestinal segment, and this segment's influence on digesta passage through the GI-tract. Figure 3 gives a schematic

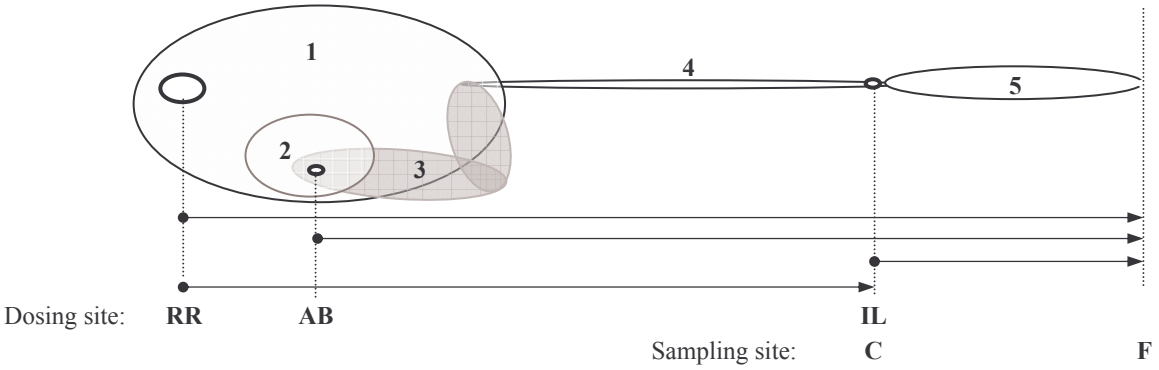

Figure 3. Schematic representation of the GI-tract with 1 = reticulo-rumen; 2 = omasum; 3 = abomasum; 4 = small intestine; 5 = caecum and large intestine. RR, AB and IL respectively depict the ruminal, abomasal and ileal pulse dose sites. Samples were collected at the ileum (C) and after defaecation (F).

Table 5. Mean values for the TT, CMRT1, CMRT2 and TMRT for different markers after pulse dosing into the ileum, abomasum and rumen, averaged for all experimental data.[1,2]

Marker	N	TT (se)	CMRT1 (se)	CMRT2 (se)	TMRT (se)
Ileal pulse dose, faecal sampling (IL)					
Co	17	3.72 (0.35)	2.11 (0.19)	1.11 (0.09)	6.95 (0.50)
Cr	20	2.52 (0.23)	2.82 (0.32)	0.58 (0.04)	5.93 (0.36)
^{13}CDM	17	1.94 (0.29)	4.53 (0.85)	0.50 (0.05)	6.03 (0.28)
^{13}CNDR	5	2.73 (0.59)	3.09 (0.42)	0.59 (0.07)	6.94 (0.74)
^{13}CNDS	5	2.41 (0.52)	3.24 (0.58)	0.55 (0.12)	6.89 (0.55)
Abomasal pulse dose, faecal sampling (AB)					
Co	20	6.25 (0.32)	3.08 (0.35)	1.06 (0.03)	10.39 (0.42)
Cr	20	7.14 (0.31)	5.78 (0.93)	1.00 (0.12)	13.91 (1.02)
^{13}CDM	20	6.29 (0.54)	4.85 (0.58)	0.85 (0.13)	11.99 (0.75)
^{13}CNDR	6	6.73 (0.81)	5.49 (1.13)	1.02 (0.11)	13.23 (1.30)
^{13}CNDS	6	6.45 (0.97)	7.13 (2.45)	0.90 (0.28)	14.48 (1.89)
Ruminal pulse dose, faecal sampling (RF)					
Co	20	8.94 (0.59)	8.69 (0.45)	2.19 (0.19)	19.81 (0.69)
Cr	20	13.77 (0.65)	19.30 (0.82)	4.10 (0.23)	37.17 (1.18)
^{13}CDM	19	15.84 (0.63)	32.66 (2.81)	8.09 (0.38)	56.59 (2.60)
^{13}CNDR	19	21.53 (1.34)	59.24 (6.62)	10.43 (0.50)	91.20 (6.46)
^{13}CNDS	19	15.93 (0.94)	30.11 (3.27)	6.94 (0.55)	52.98 (2.62)
Ruminal pulse dose, ileal sampling (RI)					
Co	20	3.73 (0.43)	7.80 (0.39)	1.08 (0.03)	12.61 (0.39)
Cr	20	7.95 (0.65)	19.90 (1.05)	2.74 (0.26)	30.58 (0.86)
^{13}CDM	20	13.11 (1.10)	27.02 (1.59)	8.07 (0.77)	48.20 (1.83)
^{13}CNDR	19	15.65 (1.15)	51.81 (6.42)	8.93 (0.65)	76.40 (5.31)
^{13}CNDS	19	9.87 (0.86)	27.33 (1.89)	5.38 (0.55)	42.58 (1.21)

[1] Averages expressed as arithmetical means with standard errors (se) between parenthesis.
[2] TT = transit time, (h); CMRT1 = residence time in the slowest compartment, (h); CMRT2 = residence time in the second slowest compartment, (h); TMRT = TT + CMRT1 + CMRT2, (h)

representation of the modified animal's intestinal tracts. The similarities in treatments between experiments (marker types, sites of pulse dosing and sampling) facilitate a comparison over and between experiments. To further improve this, all excretion patterns, including those following from ileal and abomasal pulse dosing, have been analysed using the MC-model (Dhanoa *et al.*, 1985). Table 5 summarises the transit times and the compartmental residence times, associated with the subsequent excretion curves. Following from a pulse dose in the ileum, the observed values for TT_{IL}, $CMRT1_{IL}$ and $CMRT2_{IL}$ suggest only minor differences in passage behaviour between markers (different shape of curves). The resulting total mean residence time in this compartment ($TMRT_{IL}$) does not show any difference between markers and averages between 6 and 7 hours. In this compartment the TT reflects the

longitudinal propulsion of the digesta, the CMRT1 resembles the slowest mixing compartment (caecum) and the CMRT2 the second slowest compartment (large intestine). With regard to the particle phase markers this would imply that the larger part of the $TMRT_{IL}$ (45 to 75%) relates to the mixing action in the caecum whilst about 8 to 10% of the time is related to mixing in large intestine.

After introduction of markers into the abomasum, the TT_{AB}, $CMRT1_{AB}$ and $CMRT2_{AB}$ hypothetically could reflect respectively the post-rumen digesta propulsion (i), the caecum-large intestine mixing compartments (ii) and the mixing action in the abomasum (iii). The post-rumen total mean residence time ($TMRT_{AB}$) ranges from 10.4 (Co) to 14.5 (^{13}CNDS) h, which results in a residence time in the abomasum-small intestine compartment ($TMRT_{A+SI}$ = $TMRT_{AB}$ - $TMRT_{IL}$) of about 3 h for Co, 8 h for Cr and 6 to 7 h for the internal markers. With a residence time in the abomasum ($CMRT2_{AB}$) that averages between 50 to 65 min for all markers, the residence time in the small intestine would be 2 h for Co, 7 h for Cr and 5 to 6 h for ^{13}C-markers. These results match with earlier observations of O'Connor *et al.* (1984). In an experiment with two ileum and duodenum cannulated steers they observed large animal differences in large intestinal residence times (5.3 to 13.9 h) and post-rumen residence times (8.0 to 14.5 h). Based on a slaughter trial the same authors determined abomasal residence times of 1.3 h, 8 to 9 h in the small intestine (difference between abomasum and caecum sac) and about 3.5 h in the caecum (difference between caecum sac and caecum canal).

When comparing the faecal and ileal excretion patterns of rumen pulse dosed Cr-NDF, ^{13}CDM and ^{13}CNDR, our data indicate linearity between estimates for the ileal $CMRT1_{RI}$ and the faecal $CMRT1_{RF}$ (Figure 4a). However, large variation resulted in low coefficients of determination of 38, 12 and 28% for the respective markers. Considering the curve fit interpretations of Dhanoa *et al.* (1985), the CMRT1 should represent the residence times in the rumen and one would expect that the ileal and faecal derived residence times were stronger correlated. The difference between the faecal and ileal determined TT (TT_{RF}–TT_{RI}) should correct for the time delay related to the longitudinal propulsion in the large intestine compartment. Our results show that the TT based on this calculation is 0.8 to 3.6 h longer compared to the time delay determined after an ileum pulse dose (TT_{IL}). A possible explanation could be that when materials are introduced into the ileum they by-pass the caecum and directly move on to the large intestine. A comparison between the $TMRT_{IL}$ and the $CMRT2_{RF}$ (Figure 4b) shows that the model estimates of the latter result in more variability in residence times between markers than those obtained after an ileum pulse dose. It remains, however, difficult to determine whether the CMRT2 indeed truly reflects the residence time in the caecum-large intestine compartment or that the direct measurements (i.e., after an ileum pulse dose) give the most accurate information. The $TMRT_{C+LI}$ can also be calculated from the difference between the faecally and ileally determined TMRT after marker introduction in the rumen ($TMRT_{faecal}$–$TMRT_{ileal}$). Regression of these data on the $TMRT_{IL}$ (Figure 4c) shows that especially the ^{13}CDM and ^{13}CNDR give considerable longer values for $TMRT_{C+LI}$ when derived from the difference between faecal and ileal excretion.

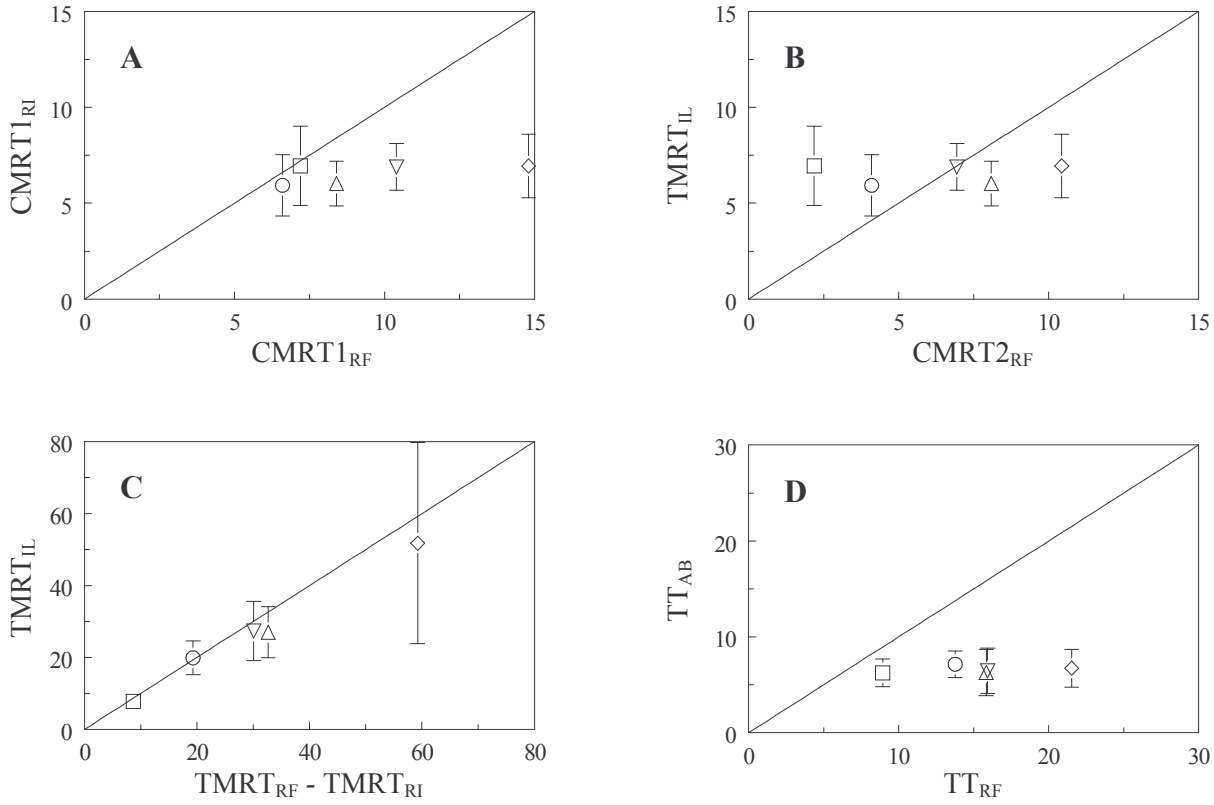

Figure 4. Relations between different estimations on compartmental residence times, with (**A**) the relation between the faecal and ileal determined CMRT1, (**B**) the relation between the faecal determined CMRT2 and the TMRT after ileal pulse dosing, (**C**) the relation between TMRT after pulse dosing and the calculated large intestininal residence time, and (**D**) the relation between faecal determined TT and the TT after abomasum pulse dosing. Different symbols represent the average residence times for cobalt (□); chromium (○), ^{13}CDM (Δ),^{13}CNDR (◊), and ^{13}CNDS (∇).

This would suggest that the caecum-large intestine compartment has the ability to selectively retain the ^{13}C-marked fractions whilst the external markers are less sensitive to this.

The faecally determined time delay following from marker introduction in the rumen is regarded to represent the longitudinal transition though the tubular sections of the GI-tract (Grovum and Williams, 1973; O'Connor *et al.* 1984; Pond *et al.*, 1988). Data presented in Table 5 suggest that when markers are introduced into the abomasum, the values of TT do not indicate any interaction between marker type and marker passage behaviour through the post-rumen section of the GI-tract. This agrees with observations of Wylie *et al.* (2000), who pulse dosed liquid and particle phase markers into the abomasum of dairy cows. In contrast, the TT_{RF} gives distinct higher estimates for the time delay and shows considerable differences between markers (Figure 4d). This implies that it is questionable whether the time delay estimations by the MC-model (TT_{RF}) may be solely assigned to the longitudinal displacement of digesta in the tubular sections. After correcting the TT_{RF} for the TT_{AB}, the absolute values suggest considerable differences between markers; 2.7 h (Co), 6.6 h (Cr), 9.6 h (^{13}CDM), 14.8 (^{13}CNDR) and 9.5 h (^{13}CNDS). Hence, depending on the type of marker a different part of the time delay is likely related to other than the longitudinal displacement in the tubular sections, i.e., processes associated with events occurring in compartments cranial to the abomasum.

However, when scaling these values to the $TMRT_{RF}$ it shows that particle phase markers (internal and external) relatively spend between 16.2 to 17.9% of the $TMRT_{RF}$ in this non-tubular section of the GI-tract, which is likely to be caused by events occurring in the rumen. Considering these results in combination with the $CMRT1_{RF}$ and $CMRT2_{RF}$ it appears that the liquid phase spends about 32% of its $TMRT_{RF}$ in the abomasum-small intestine compartment. In case of Cr about 20% of the $TMRT_{RF}$ can be related to this compartment, and ^{13}CDM, ^{13}CNDR and ^{13}CNDS remain in this compartment for 11, 7 and 12% respectively.

Compartmentalisation in relation to animal and feed characteristics. Through time, many studies have been conducted to assess the influence of dietary and animal related factors on passage characteristics from the rumen of dairy cows. With regard to dietary treatments the level of feed intake (Colucci *et al.*, 1990; Tamminga *et al.*, 1989; Robinson *et al.*,1987), the quality and type of roughage (Lund, 2002; Rinne *et al.*, 1997; Bosch and Bruining, 1995; Mambrini and Peyraud, 1994), the level and the proportion of concentrates in the diet (Bosch *et al.*, 1992; Gasa *et al.*, 1991; Colucci *et al.*, 1990) have been investigated. Animal related factors of influence are stage of lactation (Lund, 2002; Bosch *et al.*, 1992; Rothfuss *et al.*, 1997; Pellikaan *et al.*, this thesis), differences in genetic merit (e.g. the potential for milk fat quantity; (Murphy *et al.*, 2000), and differences between species and breeds (Molina *et al.*, 2001; Bartocci *et al.*, 1997; Kennedy, 1982). However, comparative studies between species and dairy breeds in relation to digesta kinetics are limited. The robustness of the equations describing the rumen outflow rates, depends on in-between experimental variation, e.g., the type of markers, the site of sampling, and the mathematical models (Offer and Dixon, 2000; Beauchemin and Buchanan-Smith, 1989), but also the type of cannulae (Harmon and Richards, 1997; Faichney, 1975), method of marker administration (e.g., oral vs. intra-ruminal), and the moment of marker introduction relative to feeding time (Poore *et al.*, 1991; Siciliano-Jones and Murphy, 1986). In the animal trials described in the former chapters (2, 3, 4), the animals were subjected to the same type of surgery using the same type of cannulae, and received similar types of markers (internal and external) via similar methods of administration. The method and frequency of sample collection was identical and the marker excretion patterns were analysed using the same model (Dhanoa *et al.*, 1985) and initial settings to solve the iterative process. These conditions omitted some of the important constraints mentioned above, and hence, allow a comparison between experiments. Table 6 (A, B) presents the correlation matrix of some animal and feed characteristics in combination with the compartmental residence times of different markers derived from faecal excretion patterns upon pulse patterns into the rumen. The daily DM intake (DMI) was positively correlated to body weight (BW) (Table 6a, $P = 0.008$), whilst DM digestibility (DMd) was negatively correlated to BW ($P = 0.044$), hence, within our experimental settings animals with higher BW had higher DMI and lower DMd. However, direct comparison between DMI and DMd showed no correlation ($P = 0.792$), suggesting that factors other than DMI, affected DMd. Roughage components (NDF, ADF, ADL) were in line with the expectations negatively correlated to DMd, but the effect was non-significant ($P \geq 0.103$). The proportion

Table 6. A: Correlation matrix for factors included in the multiple regression equation, specified for animal and diet characteristics, and external markers.[1]
B: Correlation matrix for the parameters involved in the multiple regression equation, specified for internal markers.[1]

A	Animal and feed characteristics							Cobalt				Chromium			
Item	N	BW	DMI	C-ratio	NDF	ADF	ADL	FPCM	TT	CMRT1	CMRT2	TMRT	TT	CMRT1	CMRT2	TMRT
CMRT1									-0.24				0.14			
CMRT2									0.49*	-0.34			0.58**	-0.22		
TMRT									0.83***	0.30	0.47†		0.76***	0.73***	0.37	
BW									-0.17	-0.02	-0.38	-0.26	-0.31	-0.08	-0.56**	-0.34
DMI	20	0.58**							-0.57*	-0.19	-0.67**	-0.77***	-0.83***	-0.22	-0.58**	-0.73***
C-ratio	20	0.36	0.09						-0.11	0.34	-0.08	0.10	0.08	-0.32	0.06	-0.17
NDF	20	-0.09	-0.17	-0.26					-0.15	0.10	0.12	-0.03	0.07	0.14	0.32	0.20
ADF	20	-0.15	-0.22	-0.32	1.00***				-0.12	0.09	0.16	0.00	0.10	0.15	0.35	0.23
ADL	20	-0.17	-0.30	-0.17	0.98***	0.98***			-0.10	0.16	0.23	0.07	0.20	0.08	0.44†	0.25
FPCM	20	0.10	0.37	-0.33	-0.22	-0.19	-0.25		0.07	-0.52†	0.05	-0.41	-0.28	-0.27	-0.13	-0.50*
DMd	19	-0.47*	0.06	-0.66**	-0.36	-0.30	-0.39	0.58*	0.02	-0.46†	0.01	-0.31	-0.10	-0.17	-0.03	-0.22

B	^{13}CDM				^{13}CNDR				^{13}CNDS			
Item	TT	CMRT1	CMRT2	TMRT	TT	CMRT1	CMRT2	TMRT	TT	CMRT1	CMRT2	TMRT
CMRT1	-0.31				0.04				-0.58**			
CMRT2	0.37	-0.36			0.08	0.70*			0.38	-0.51*		
TMRT	-0.04	0.95***	-0.15		0.26	0.97***	0.77**		-0.36	0.96***	-0.31	
BW	-0.22	-0.16	0.29	-0.19	0.02	0.33	0.10	0.30	-0.36	-0.10	0.19	-0.18
DMI	-0.45†	-0.46*	0.14	-0.59**	-0.59*	-0.01	-0.13	-0.16	-0.07	-0.49*	0.24	-0.56*
C-ratio	0.34	-0.29	0.64**	-0.14	-0.07	0.35	0.23	0.32	0.22	-0.15	0.50*	-0.03
NDF	0.16	0.35	0.20	0.44†	0.44	-0.19	-0.08	-0.08	-0.12	0.23	0.19	0.28
ADF	0.15	0.38	0.15	0.47*	0.46	-0.21	-0.09	-0.09	-0.12	0.26	0.15	0.31
ADL	0.24	0.39	0.25	0.51*	0.49	-0.23	-0.10	-0.11	-0.07	0.30	0.21	0.38
FPCM	-0.47*	-0.04	-0.33	-0.20	-0.12	-0.25	-0.32	-0.31	-0.19	-0.11	-0.27	-0.23
DMd	-0.41†	0.10	-0.65**	-0.09	-0.34	-0.46	-0.32	-0.52†	-0.11	0.11	-0.53*	0.00

[1] n = observations; BW = body weight; DMI = daily DMI; C-ratio = concentrate ratio; NDF, ADF, ADL = fiber fractions in roughage; FPCM = fat and protein corrected milk; TT = transit time; CMRT1, CMRT2 = residence time in the slowest, and second slowest compartment; TMRT = total tract residence time.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$.

of concentrate in the diet (C-ratio) was negatively correlated to the DMd ($P = 0.002$), which agrees with literature (Colucci *et al.*, 1990; Owens and Goetsch, 1986). The positive correlation between FPCM and DMd ($P = 0.014$) suggests that DMd increases with increased milk production. A causal factor here would be the level of DMI, but although the correlation between DMI and FPCM are positive (0.37) the effect was non-significant ($P = 0.149$).

With regard to the correlations obtained for the transit times (TT), the compartmental residence times (CMRT1, CMRT2) and the total tract residence times (TMRT) considerable differences were found between markers In case of Co and Cr the TT was positively correlated to the CMRT2 and the TMRT (Table 6a, $P \leq 0.045$). The TMRT of Co and Cr was positively correlated to the CMRT1 and CMRT2, but only in case of the CMRT1 of Cr the correlation coefficient was significant (0.73, $P < 0.001$). With regard to the internal markers ^{13}CDM, ^{13}CNDR and ^{13}CNDS high correlation coefficients were observed between the CMRT1 and the TMRT (≥ 0.95, $P < 0.001$). For ^{13}CNDR a positive correlation was found between CMRT2 and TMRT ($P = 0.004$), but in case of ^{13}CDM and ^{13}CNDS the correlations were negative ($P \geq 0.204$). The TT-values of the latter two were negatively correlated to the CMRT1 ($P \geq 0.012$) and TMRT ($P \geq 0.140$), but positively correlated to CMRT2 ($P \geq 0.124$).

With regard to the residence times (TT, CMRT1, CMRT2, TMRT) of Co and Cr, DMI showed distinct negative correlations ($P \leq 0.474$). The internal markers showed in general similar, but less pronounced correlations. However, for the CMRT2 of ^{13}CDM and ^{13}CNDS non-significant but positive correlations were observed with regard to DMI. In relation with the remaining observed correlations, the CMRT1 of Co was negatively correlated to FPCM ($P = 0.055$) and DMd ($P = 0.071$). In case of Cr, the CMRT2 was positively related to the ADL content of the roughage ($P = 0.052$) and TMRT was negatively correlated to FPCM ($P = 0.043$). For ^{13}CDM positive relations were observed between CMRT2 and C-ratio ($P = 0.003$) and between TMRT and NDF, ADF and ADL ($0.059 \geq P \leq 0.025$). ^{13}CNDS showed similar but less pronounced correlations and ^{13}CNDR showed contrasting results. In summary, these data suggest that residence times of the external markers Co and Cr are largely correlated to DMI and to a lesser extent to the other factors (C-ratio, NDF, ADF, ADL, FPCM, DMd). The internal markers show similar, but lower correlations for DMI and relatively larger correlation coefficients for the other diet and animal related factors, particularly in case of ^{13}CDM. This would indicate that ^{13}C marked materials form a potential tool to quantify the influence of diet and animal related factors on the residence times in the different compartments of the animals GI-tract.

Table 7 summarises the relations between some animal and feed related characteristics, and the compartmental residence times of the different markers used in our experiments. Relations were analysed using the linear regression procedure in SAS (PROC REG, SELECTION=CP; SAS Inst. Inc., Cary, NC, 1996), where BW, DMI, C-ratio, the NDF, ADF and ADL of roughage, DMd and FPCM were included as independent variables. The criteria to include variables in the model were a combination of; a low value for the Mallow's C*p*-criterion, a

Table 7. The relations between some animal and diet characteristics, and the compartmental residence times of different markers estimated by multiple linear regression.[1]

Marker	n	α	BW	DMI	C-ratio	NDF[2]	ADF[2]	ADL[2]	DMd	FPCM	R^2
Transit time (TT)											
Co	14	1.5^{NS}		-0.60^{NS}		0.128^{NS}		-1.780^{NS}			0.183
Cr	17	-9.5^{NS}	0.034^{*}	-1.29^{***}	9.1^{NS}				26.3^{*}		0.753
^{13}CDM	17	161.2^{**}	-0.058^{**}			0.582^{NS}	-1.31^{NS}		-25.5^{NS}		0.625
^{13}CNDR	11	10.3^{NS}	0.054^{*}	-1.42^{*}							0.585
^{13}CNDS	16	$120.5^{\dagger}$	$-0.069^{\dagger}$					$-0.432^{\dagger}$	$-85.9^{\dagger}$	0.322^{NS}	0.332
Mean residence time slowest compartment (CMRT1)											
Co	14	7.6^{NS}	0.008^{NS}		4.0^{NS}					-0.226^{NS}	0.376
Cr	17	149.4^{***}			-62.3^{***}			-0.623^{**}	-113.4^{**}	-0.177^{NS}	0.699
^{13}CDM	17	-452.5^{*}	0.212^{*}	-4.40^{*}		-2.471^{*}	5.69^{*}				0.599
^{13}CNDR	11	405.9^{**}						-2.536^{*}	-386.0^{**}		0.628
^{13}CNDS	16	-151.0^{NS}	$0.215^{\dagger}$	-6.65^{*}				0.604^{NS}	199.8^{NS}		0.509
Mean residence time second slowest compartment (CMRT2)											
Co	14	$-20.1^{\dagger}$			6.2^{NS}	$0.012^{\dagger}$			$18.3^{\dagger}$		0.361
Cr	17	$-206.1^{\dagger}$			$45.9^{\dagger}$	-0.738^{*}	$2.09^{\dagger}$	-1.957^{NS}			0.673
^{13}CDM	17	22.4^{**}		1.04^{***}		-0.179^{***}		2.387^{***}		-0.230^{**}	0.801
^{13}CNDR	11	6.9^{NS}		0.53^{NS}						-0.259^{NS}	0.282
^{13}CNDS	16	$21.1^{\dagger}$		1.31^{**}		-0.208^{**}		2.850^{**}		-0.312^{*}	0.685
Total mean residence time (TMRT)											
Co	14	17.4^{*}	0.023^{NS}	-0.56^{NS}						-0.123^{NS}	0.379
Cr	17	48.5^{***}	0.047^{*}	-0.98^{*}	-28.9^{**}					-0.525^{*}	0.688
^{13}CDM	17	-74.1^{NS}	$0.163^{\dagger}$	-5.08^{*}				1.265^{NS}	116.2^{NS}		0.590
^{13}CNDR	11	-4.4^{NS}	0.227^{*}							$-1.593^{\dagger}$	0.587
^{13}CNDS	16	-1123.0^{*}	0.110^{NS}		197.7^{**}	-5.770^{**}	13.33^{**}				0.583

[1] n = no. of observations; α = intercept; BW = body weight (kg); DMI = daily dry matter intake (kg/day); C-ratio = amount of compound feed in diet; DMd = dry matter digestibility (%); FPCM = fat and protein corrected milk (kg); R^2 = coefficient of determination.

[2] NDF, ADF, ADL = determined in the roughage component.

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$; ^{NS}, non significant.

high coefficient of determination (R^2), and setting the residual degrees of freedom (df) in the regression model at >65% of the total df.

Cr gave in general good results with R^2-values ranging between (0.67 to 0.75), followed by ^{13}CDM, that gave lower R^2-values for the TT, CMRT1 and TMRT (0.59 to 0.63) but higher in case of the CMRT2 (0.80). The R^2-values for Co were in all cases low and suggest that the compartmental residence times of Co cannot be explained by the animal and feed characteristics included in Table 7. With regard to the TT, Cr and ^{13}CNDR showed significant positive and negative regression coefficients for respectively BW and DMI. In contrast, the TT of ^{13}CDM and ^{13}CNDS was negatively related to BW whilst DMI showed no relation. The negative influence of DMI on the TT-values obtained for Co, Cr and ^{13}CNDR seem

biologically rational. However, negative regression coefficients for ADF and ADL in combination with positive coefficients for NDF (Co and ^{13}CDM) are difficult to interpret. A change in sign would suggest considerable variation in the ratios ADF/NDF and ADL/NDF between experimental treatments. Although roughage quality and dietary treatments between sucessive experiments varied, the variation in total diet ADF/NDF-ratio ranged 0.595 to 0.632. ADL/NDF ratio ranged from 0.05 to 0.08. The major part of this difference was likely explained by the contrasts in roughage quality, in combination with a lower proportion of concentrates in the trial with fresh grass fed animals. The negative relations between DMI and the CMRT1 of ^{13}CDM and ^{13}CNDS agree with literature observations (Bosch *et al.*, 1992; Colucci *et al.*, 1990; Tamminga *et al.*, 1989). The negative relations with FPCM of Co and Cr, match the expectations and are likely confounded with the DMI. Although DMI and FPCM were positively related (0.37, Table 6) the correlation coefficient was non-significant. An increase in NDF and ADL resulted in a decrease in CMRT1 (higher passage rate), which corresponds with observations of Rinne *et al.* (1997), Bosch and Bruining (1995) and Gasa *et al.* (1991). In case of Cr and ^{13}CNDR the CMRT1 was negatively related to the DMd (Table 6), implying that diets with a higher overall apparent DM digestibility give higher fractional rates of passage for these markers (shorter CMRT1). This agrees with observations of Lund (2000) where animals in late lactation received early cut (OMd = 76.3%) and late cut (OMd = 61.4%) grass silage, but contrasts with the findings of Rinne *et al.* (1997), Mambrini and Peyraud (1994) and Bosch *et al.* (1992). The amount of concentrate in the diet had a significant negative influence on the CMRT1 of Cr, but in the correlation matrix (Table 6) the effect was minor. In general, an increase in the amount of concentrate in the diet will result in a decrease of the fractional particle outflow from the rumen (Bartocci *et al.*, 1997; Huhtanen and Jaakkola, 1993; Bosch *et al.*, 1992; Colucci *et al.*, 1990). However, the latter authors also observed that passage kinetics of different particle phase markers and liquid phase markers were differently affected by the concentrate ratio in a diet when animals were subjected to higher or lower levels DMI.

The residence times of the internal markers in the second slowest compartment (CMRT2) was negatively related to FPCM (Table 7), which agrees with observations of Molina *et al.* (2001) who studied passage kinetics in different breeds of dairy sheep at different stages of lactation. In their study milk production of both breeds decreased with stage of lactation, however, only one breed significantly dropped in daily DMI with time. In the current study (Table 7) daily DMI was positively related to the CMRT2, which seems to be contradictory. Corresponding correlation coefficients (Table 6) proved however, that correlations were poor. Although correlation coefficients for Co (-0.67) and Cr (-0.58) between DMI and CMRT2 were significant, the effect did not significantly contribute in the multiple regression analyses. Hence, DMI was not included in the equations for Co and Cr in Table 7. Between the concentrate ratio in the diet (C-ratio) and the CMRT2, significant correlation coefficients were found for ^{13}CDM (0.64) and ^{13}CNDS (0.50) (Table 6B). However, C-ratio did not significantly contribute in the multiple regression analyses, and hence, was therefore not

included in the equation (Table 7). The total tract mean residence time (TMRT) of all markers were positively related to BW with the biggest impact of BW on the TMRT of ^{13}CDM and ^{13}CNDR. However, the importance of this relation is difficult to value. The negative relations between the TMRT and DMI are in line with regression coefficients in equations presented by Van Straalen (1995), however, in this study DMI was the sole determining variable included in the model.

From the information based on the current experiments it may be concluded that the height of the residence times of particle phase markers corresponding to different mixing compartments (CMRT1, CMRT2) or other than mixing compartments (TT), can be reasonably well related to some animal en feed characteristics that are easily available in on farm situations. Due to the limitations in the experimental designs (small number of animals in combination with a range of dietary treatments), relations should only be regarded within the experimental conditions, and hence, extrapolation to situations outside the range (e.g., in case of high producing/ high intake animals) should be done cautiously.

Relations between fractional rate constants, animal and feed characteristics. Through time, many studies have been conducted to assess the influence of dietary and animal related factors on fractional passage of liquid and solid associated markers (Van Straalen, 1995; Pitt *et al.*, 1992; Sniffen *et al.*, 1992; Bosch; 1991; Owens and Goetsch, 1986). Table 8 summarises the equations and concomitant regression coefficients. Our observed fractional passage rates from the rumen compartment have been compared with some of the predicted

Table 8. Factors contributing to fractional rate constants describing outflow from the rumen as reported in literature.

Passage[1]	Notation[3]	R^2	Source
Fluid	$1.059 + 0.0458 \cdot (DMI/BW)$	-	Pitt *et al.*, 1996
Fluid	$-3.40 + 1.224 \cdot DMI - 0.03 \cdot DMI^2 + 5.93 \cdot \%R$	0.41	Van Straalen, 1995
Roughage	$1.743 + 0.149 \cdot DMI$	0.55	Van Straalen, 1995
Concentrate	$10.08 - 0.963 \cdot DMI + 0.037 \cdot DMI^2$	0.55	Van Straalen, 1995
Forage[2]	$0.38 + 0.022 \cdot (DMI/MW) + 0.0002 \cdot \%R$	-	Sniffen *et al.*, 1992
Concentrate	$-0.424 + 1.45 \cdot K_{(forage)}$	-	Sniffen *et al.*, 1992
DM	$14.4 - 0.0018 \cdot FCM - 0.008 \cdot NDFR + 0.003 \cdot \%C - 0.007 \cdot BW$	0.68	Bosch, 1991[4]
Fluid	$4.12 + 0.77 \cdot CI + 2.32 \cdot RI$	0.29	Owens & Goetsch, 1986
Roughage	$0.94 + 1.34 \cdot CI + 1.24 \cdot RI$	0.38	Owens & Goetsch, 1986
Concentrate	$1.30 + 0.61 \cdot CI + 4.88 \cdot RI - 1.25 \cdot RI^2$	0.61	Owens & Goetsch, 1986

[1] rate constants for different fractions and phases in %/h.

[2] corrected regression coefficients adopted from Offer and Dixon (2000).

[3] DMI = daily total DM intake (kg/d); %R = proportion of roughage in the diet; %C = proportion of concentrate in the diet; FCM = fat corrected milk (kg/d); BW = body weight (kg); MW = metabolic weight (kg); $K_{(forage)}$ = fractional passage of forages estimated by Sniffen *et al.*, 1992 (%/h); CI = the amount of concentrate intake scaled to BW (%); RI = the amount of roughage intake scaled to BW.

[4] Equation describes total rumen clearance of DM.

rumen outflow rates estimated by the equations in Table 8. Figure 5 presents estimates for markers based on equations of Van Straalen (**A**), Owens and Goetsch (**B**), and Sniffen *et al.* (**C**), and estimates of liquid outflow by various authors (**D**). Comparing the estimations for particle phase markers differences between A, B and C are small and hardly explain the observed variation. The liquid passage estimated by the Owens and Goetsch equation (B and D), in general underestimates the actual values, whilst predictions by the equation of Van Straalen (A) tend to higher values. The equation of Pitt *et al.* shows good results to fractional rates of about $10\%^{-h}$, but also fails to accurately describe the observed higher rate constants. In general the coefficients of determination are poor and in itself already explain that adopting passage rates based on such equations leave considerable room for error in estimating nutrient supply to the animal. Our equations presented in Table 7, in some occasions give higher R^2 suggesting more accurate fits. However, the experimental conditions were explicit. In analogy, Figure 4 indicates that these conditions allow for variability in the 'observed situation' that was not picked-up by the equations from literature. In these equations the

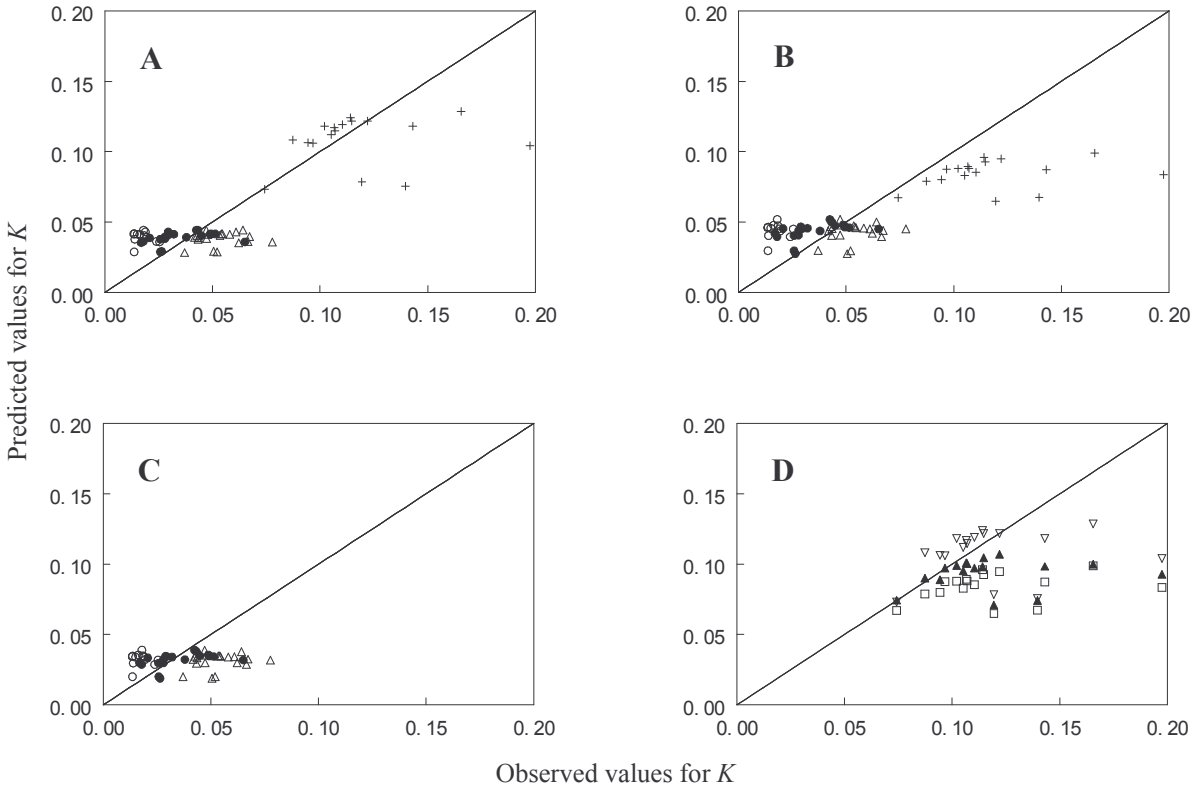

Figure 5. Comparison of the observed and predicted values for the fractional passage rates for different fractions, where + = Co; Δ = Cr; ● = ^{13}CDM; ○ = ^{13}CNDR. (**A**) equations of Van Straalen, 1995; (**B**) equations from Owens & Goetsch, 1986; (**C**) equations from Sniffen *et al.* 1992.
(**D**) reflects the fluid passage for different authors; where ∇ = Van Straalen, 1995; ▲ = Pitt *et al.*, 1996; □ = Owens & Goetsch, 1986.

regressors included into the equations are limited to intake parameters (absolute or relative to body weight) and in some occasions the amount and ratio of concentrate in the diet is regarded an important factor. Hence, other animal and feed related factors that could contribute to the dynamics of digesta passage are excluded. In accord with earlier observations of Bosch (1991) it would be desirable to obtain more information on possible related factors in order to include them in such equations.

Literature Cited

AFRC. 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. AFRC Technical Committee on Responses to Nutrients. Report no. 11. Nutr. Abstr. Rev. (Ser. B) 68: 937-989.

Aitchison, E., M. Gill, J. France, and M. S. Dhanoa. 1986. Comparison of methods to describe the kinetics of digestion and passage of fibre in sheep. Journal of the Science of Food and Agriculture. 37(11):1065-1072.

Amici, A., S. Bartocci, S. Terramoccia, and F. Martillotti. 1997. Passage rate of solids and fluids in the digestive tract of buffaloes, cattle and sheep: selection of non-linear model. Animal Science. 64(1):63-69.

Armsby, H. P. 1914. The principles of animal nutrition, with special reference to the nutrition of farm animals. Wiley and Sons, Inc., New York.

Balch, C. C. 1950. Factors affecting the utilization of food by dairy cows 1. The rate of passage of food through the digestive tract. Brit. J. Nutr. 4:361-387.

Bartocci, S., A. Amici, M. Verna, S. Terramoccia, and F. Martillotti. 1997. Solid and fluid passage rate in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livest. Prod. Sci. 52(3):201-208.

Beauchemin, K. A. and J. G. Buchanan Smith. 1989. Evaluation of markers, sampling sites and models for estimating rates of passage of silage or hay in dairy cows. Anim. Feed Sci. Technol. 27(1-2):59-75.

Bernard, L., J. P. Chaise, E. Delval, and C. Poncet. 1998. Validation of the main modeling methods for the estimation of marker mean retention times in the different compartments of the gastrointestinal tract in sheep. J. Anim. Sci. 76:2485-2495.

Bibby, J. and H. Toutenburg. 1977. Prediction and improved estimation in linear models.

Blaxter, K. L., N. M. Graham, and F. W. Wainman. 1956. Some observations on the digestibility of food by sheep, and on related problems. Brit. J. Nutr. 10:69-91.

Bosch, M. 1991. Influence of stage of maturity of grass silages on digestion processes in dairy cows. Thesis, Wageningen University.

Bosch, M. W. and M. Bruining. 1995. Passage rate and total clearance rate from the rumen of cows fed on grass silages differing in cell-wall content. Brit. J. Nutr. 73(1):41-49.

Bosch, M. W., S. C. W. Lammers-Wienhoven, G. A. Bangma, H. Boer, and P. W. M. van Adrichem. 1992. Influence of stage of maturity of grass silages on digestion processes in dairy cows. 2. Rumen contents, passage rates, distribution of rumen and faecal particle and mastication activity. Livest. Prod. Sci. 32(3):265-281.

Campling, R. C. and M. Freer. 1962. THe effect of specific gravity and size on the mean time of retention of inert particles in the alimentary tract of the cow. Brit. J. Nutr. 16:507-518.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, I. McMillan, and D. J. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 73(8):2143-2156.

de Greeve, P. C. M., J. Hampson, and L. F. M. van Zutphen. 1993. Legislation and animal experimentation. Pages 9-16 *in* Principles of laboratory animal science. A contribution to the human use and care of animals and to the quality of experimental results. L. F. M. van Zutphen, V. Baumans, and A. C. Beynen, eds. Elsevier Science Publishers B.V., Amsterdam.

Dhanoa, M. S., J. France, and R. C. Siddons. 1989. On using a double-exponential model for describing faecal marker concentration curves. Journal of Theoretical Biology. 141:247-257.

Dhanoa, M. S., R. C. Siddons, J. France, and D. L. Gale. 1985. A multicompartmental model to describe marker excretion patterns in ruminant faeces. Brit. J. Nutr. 53(3):663-671.

Faichney, G. J. 1975. The use of markers to partition digestion within the gastro-intestinal tract of ruminants. Pages 277-291 *in* Digestion and metabolism in Ruminants. Vol. -. 33 ed. I. W. McDonald and A. C. I. Warner, eds. The university of New England, Armidale.

France, J. and J. H. M. Thornley. 1984. Mathematical models in agriculture. Butterworths, London, UK.

France, J., J. H. M. Thornley, R. C. Siddons, and M. S. Dhanoa. 1989. Determination of rumen volume in nonsteady state using digesta markers: a dynamic, deterministic, mechanistic approach. Third international workshop on modelling digestion and metabolism in farm animals, Canterbury, New Zealand:159-168.

Gasa, J., K. Holtenius, J. D. Sutton, M. S. Dhanoa, and D. J. Napper. 1991. Rumen fill and digesta kinetics in lactating Friesian cows given two levels of concentrates with two types of grass silage ad lib. Brit. J. Nutr. 66(3):381-398.

Grovum, W. L. and G. D. Phillips. 1973. Rate of passage of digesta in sheep part 5. Theoretical considerations based on a physical model and computer simulation. Brit. J. Nutr. 30(2):377-390.

Grovum, W. L. and V. J. Williams. 1973. Rate of passage of digesta in sheep part 4. Passage of marker through the alimentary tract and the biological relevance of rate constants derived from the changes in concentration of marker in feces. Brit. J. Nutr. 30(2):313-329.

Harmon, D. L. and C. J. Richards. 1997. Considerations for gastrointestinal cannulations in ruminants. J. Anim. Sci. 75(8):2248-2255.

Henneberg, W. and F. Stohmann. 1860. Beitrage zur begruendung einer rationellen fuetterung der wiederkauwer: praktisch landwirtschaftliche und chemisch-physiologische untersuchungen fuer landwirthe und physiologen. Schmetschke und Sohn, Braunschweig.

Huhtanen, P. and A. N. Hristov. 2001. Estimating passage kinetics using fibre-bound N-15 as an internal marker. Anim. Feed Sci. Technol. 94(1-2):29-41.

Huhtanen, P., and S. Jaakkola. 1993. The effects of forage preservation method and proportion of concentrate on digestion of cell wall carbohydrates and rumen digesta pool size in cattle. Grass and Forage Science 48: 155-165.

Huhtanen, P., S. Jaakkola, and U. Kukkonen. 1995. Ruminal plant cell wall digestibility estimated from digestion and passage kinetics utilizing mathematical models. Anim. Feed Sci. Technol. 52(1-2):159-173.

Huhtanen, P. and U. Kukkonen. 1995. Comparison of methods, markers, sampling sites and models for estimating digesta passage kinetics in cattle fed at two levels of intake. Anim. Feed Sci. Technol. 52(1-2):141-158.

Kennedy, P. M. 1982. Ruminal and intestinal digestion in Brahman crossbred and Hereford cattle fed alfalfa or tropical pasture hay. J. Anim. Sci. 55(5):1190-1199.

Lund, P. 2002. The effect of forage type on passage kinetics and digestibility of fibre in dairy cows. The Royal Veterinary and Agricultural University, Copenhagen.

MacRae, J. C. 1975. The use of re-entrant cannulae to partition digestive function within the gastro-intestinal tract of ruminants. Pages 261-276 *in* Digestion and metabolism in Ruminants. Vol. -. 33 ed. I. W. McDonald and A. C. I. Warner, eds. The university of New England, Armidale.

Mambrini, M. and J. L. Peyraud. 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet. Reprod. Nutr. Dev.. 34(1):9-23.

Mambrini, M. and J. L. Peyraud. 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculations based on faecal collection. Reprod. Nutr. Dev.. 37(4):427-442.

Matis, J. H. 1972. NOTE: Gamma time-dependency in Blaxter's compartmental model. Biometrics. -(-):597-602.

Matis, J. H., W. C. Ellis, and D. M. Allen. 1989. On a stochastic approach to modelling ruminant digestion and metabolism. Third international workshop on modelling digestion and metabolism in farm animals, Canterbury, New Zealand:139-158.

Molina, E., A. Ferret, C. Caja, X. Calsamiglia, X. Such, and J. Gasa. 2001. Comparison of voluntary food intake, apparent digestibility, digesta kinetics and digestive tract content in Manchega and Lacaune dairy sheep in late pregnancy and early and mid lactation. Animal Science. 72:209-221.

Moore, J. A., K. R. Pond, M. H. Poore, and T. G. Goodwin. 1992. Influence of model and marker on digesta kinetic estimates for sheep. J. Anim. Sci. 70(11):3528-3540.

Motulsky, H. J. and L. A. Ransnas. 1987. Fitting curves to data using nonlinear regression: A practical and nonmathematical review. FASEB Journal.365-374.

Murphy, M., M. Åkerlind, and K. Holtenius. 2000. Rumen fermentation in lactating cows selected for milk fat content fed two forage to concentrate ratios with hay or silage. J. Dairy Sci. 83:756-764.

O'Connor, J. D., P. H. Robinson, C. J. Sniffen, and M. S. Allen. 1984. A Gastro-intestinal tract simulation model of digesta flow in ruminants. Canadian Society of Animal Sciences, occasional publication 1.:102-122.

Offer, N. W. and J. Dixon. 2000. Factors affecting outflow rate from the reticulo-rumen. Nutrition Abstracts and Reviews. Series B, Livestock Feeds and Feeding. 70(11):833-844.

Pitt, R. E., J. S. van Kessel, D. G. Fox, A. N. Pell, M. C. Barry, and P. J. van Soest. 1996. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. J. Anim. Sci. 74(1):226-244.

Pond, K. R., W. C. Ellis, J. H. Matis, H. M. Ferreiro, and J. D. Sutton. 1988. Compartment models for estimating attributes of digesta flow in cattle. Brit. J. Nutr. 60(3):571-595.

Poore, M. H., J. A. Moore, T. P. Eck, and R. S. Swingle. 1991. Influence of passage model, sampling site, and marker dosing time on passage of rare earth-labeled grain through Holstein cows. J. Anim. Sci. 69(6):2646-2654.

Poppi, D. P., J. France, and S. R. McLennan. 1999. Intake, passage and digestibility. Pages 35-52 *in* Feeding systems and feed evaluation models. M. K. Theodorou and J. France, eds. CAB International, Wallingford.

Quiroz, R. A., K. R. Pond, E. A. Tolley, and W. L. Johnson. 1988. Selection among nonlinear models for rate of passage studies in ruminants. J. Anim. Sci. 66(11):2977-2986.

Rinne, M., P. Huhtanen, and S. Jaakkola. 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol. 67(1):19-35.

Robinson, P. H., S. Tamminga, and A. M. van Vuuren. 1987. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. Livest. Prod. Sci. 17(1):37-62.

Rodrigues, S. I. F. C., J. Dijkstra, A. G. Assis, J. France, and S. Tamminga. 2002. Evaluation of supplements to enhance nutrient supply and milk production of cattle fed diets based on elephant grass (Pennisetum purpureum) using a mathemetical model. Livest. Prod. Sci. 77:273-286.

Roordink, M. 1999. Schatten van passagesnelheden: Toepasbaarheid van niet lineaire compartiment modellen om bij melkkoeien faecale en ileale patronen van markeerstofconcentraties te beschrijven. -. Wageningen University, Wageningen.

Rothfuss, H., K. H. Sudekum, and M. Stangassinger. 1997. Estimation of marker passage in the digestive tract of dairy cows by different models: influence of stage of lactation, amount of intake and diet composition. Archives of Animal Nutrition. 50(1):43-61.

SAS-Institute. 1996. SAS/STAT software. SAS Institute Inc., Cary, NC, USA.

Savoie, P., Y. R. Agbossamey, and H. V. Petit. 2001. Retention time of macerated alfalfa hay and silage in sheep. J. Anim. Sci. (507-514).

Siciliano-Jones, J. and M. R. Murphy. 1986. Passage of inert particles varying in length and specific gravity through the postruminal digestive tract of steers. J. Dairy Sci. 69(9):2304-2311.

Sniffen, C. J., J. D. O'-Connor, P. J. van Soest, D. G. Fox, and J. B. Russell. 1992. A Net Carbohydrate and Protein System for evaluating cattle diets. 2. Carbohydrate and protein availability. J. Anim. Sci. 70(11):3562-3577.

Tamminga, S., W. J. J. Gerrits, A. Bannink, J. Dijkstra, G. A. L. Meijer, and A. M. van Vuuren. 2000. Een opzet voor een nieuw, op nutriënten gebaseerd voederwaarderingssysteem voor herkauwers. 25ste studiedag voor Nederlandstalige voedingsonderzoekers, Wageningen:20-21.

Tamminga, S., P. H. Robinson, M. Vogt, and H. Boer. 1989. Rumen ingesta kinetics of cell wall components in dairy cows. Anim. Feed Sci. Technol. 25(1-2):89-98.

Tamminga, S., W. M. van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40(2):139-155.

Uden, P., T. R. Rounsaville, G. R. Wiggans, and P. J. van Soest. 1982. The measurement of liquid and solid digesta retention in ruminants, equines and rabbits given timothy (*Phleum pratense*) hay. Brit. J. Nutr. 48(2):329-339.

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

Wolf, E. 1861. Die landwirtschaftliche fütterungslehre und die theorie der menschlichen ernährung. J.O. Gottaschen Buchhandlung, Stuttgart.

Wylie, M. J., W. C. Ellis, J. H. Matis, E. M. Bailey, W. D. James, and D. E. Beever. 2000. The flow of forage particles and solutes through segments of the digestive tracts of cattle. Brit. J. Nutr. 83(3):295-306.

Chapter 7

General Discussion

General Discussion

Introduction

Fractional passage rate constants form an essential element within modern feed evaluation systems for ruminants. Most systems for protein evaluation that are currently in use have adopted fixed values for passage in order to describe extent of protein degradation in the rumen (Tamminga *et al.*, 1994). Future developments in feed evaluation show preferences towards systems of a more mechanistic, deterministic nature (Dijkstra *et al.*, 1998). In these so-called nutrient based evaluation systems the energy and protein supply to the animal should be fully integrated entities. Moreover, knowledge on the formation of fermentation end-products and their fate in metabolic pathways should give quantitative information in the utilisation of these products for basic metabolism, for the synthesis of milk and milk components (fat, protein, lactose), and for growth and development (AFRC, 1998). In such systems, knowledge on feed or rather feed component specific residence times (the reciprocal of fractional passage) in the different compartments of the gastro-intestinal tract is essential. The underlying model equations describing the processes are sensitive to changes in passage rate constants, and hence, estimations on passage rate constants should be considered with careful attention (Rodrigues *et al.*, 2002).

Concepts of Using the Stable Isotope ^{13}C as a Marker in Passage Studies

Within the current project, information was acquired on feed (component) specific fractional passage rates through different compartments of the gastro-intestinal tract (GI-tract) of dairy cows, using the stable isotope of carbon (^{13}C) as an internal marker. In principle, all organic (carbon containing) materials in nature consist of two stable isotopic components, ^{12}C (98.89%) and ^{13}C (1.11%). Under natural conditions the ratio of these two isotopes shows only moderate variation, but differences in this ratio can be determined with high accuracy, often into the third or fourth decimal point of an atomic percentage unit (Boutton, 1991), making it a promising tool to study physiological processes. The use ^{13}C as an internal marker in nutrition research in order to follow its dynamic behaviour throughout physiological processes requires sufficient variation in the level of ^{13}C-enrichment within the nutrient of interest. In general two concepts are available to cause variation in the level of ^{13}C within a nutrient pool:

1. By addition of artificially enriched feeds or feed components.
2. By making use of the natural occurring difference in the level of enrichment between different groups of plant species (C_3 versus C_4).

Isotopic discrimination between C_3 and C_4 plants. The total plant kingdom includes some 275,000 species of which the flowering plants (angiosperms) are the most dominant with an approximate 240,000 species. The latter can be divided into two major groups according to differences in photosynthetic pathways, where each has its unique isotope fractionation pattern (O'Leary, 1988; Kimball, 2004). The majority of these plants have a C_3-photosynthetic pathway and dominate plant communities in the more temperate environments. They are often referred to as 'cool season' plants and have a relatively low level of ^{13}C-enrichment ($\delta^{13}C$ of –21 to –35 ‰ vs. PDB). In the semi-arid and arid environments plants with a C_4-photosynthetic pathway dominate the plant communities. These so-called 'warm season' plants have a distinctly higher level of enrichment ($\delta^{13}C$ of –10 to –14 ‰ vs. PDB) compared to C_3-plants (Smith and Epstein, 1971; Boutton, 1991 Ehleringer, 1991; Svejcar *et al.*, 1993; Südekum *et al.*, 1995). The term 'C_3' refers to a photosynthetic pathway in which CO_2 is reduced to 3-phosphoglyceric acid (PGA; a 3-carbon intermediate) through the enzyme ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco). C_4-plants reduce CO_2 to aspartic or malic acid (4-carbon intermediates) through the enzyme phosphoenolpyruvic carboxylase (PEP carboxylase). The enzyme Rubisco has a more profound discrimination against ^{13}C than PEP carboxylase, which explains the difference in ^{13}C-enrichment between C_3 and C_4-species (Bouton, 1991; Kimball, 2004).

Isotopic discrimination between plant fractions. Benner *et al.* (1987) reported considerable differences in isotopic enrichment between plant fractions when grown under natural conditions. In several C_3 and C_4-species they observed that the lignin fraction was depleted in $\delta^{13}C$ by 2 to 6‰-units relative to the level of enrichment of the whole-plant material. In contrast, cellulose and hemicellulose fractions proved to have a 1 to 2‰-units higher enrichment level compared to whole-plant. According to the authors the depletion of lignin had to be related to fractionation processes against ^{13}C that occur during the formation of the biosynthetic precursors of lignin, phenylalanine and tyrosine. Especially the latter is known to be relatively more depleted in ^{13}C. Because tyrosine is an important precursor for lignin in grasses, this explanation matched their observations. Our observations (Chapter 2) showed a similar but far less pronounced depletion of the lignin fraction in perennial rye grass grown under field conditions. This suggests that the ^{13}C fractionation during the biosynthesis of especially tyrosine is likely to be more variable than what is shown by Benner *et al.* (1987).
Another issue here is that when considering marker excretion patterns, it is rational to assume that the marker material collected at the beginning of a curve (e.g. at 15 h) resides for a considerable shorter time in the GI-tract then the material that is collected at a later stage (e.g. at 90 h). In consequence, it can be theorised that the material with the longer residence time will have a relatively higher proportion of lignin, and hence, a relative lower level of enrichment. This would suggest the possibility of an overestimation of the fractional rate constant that describes the declining part of the excretion curve. However, in virtually all of our observations, the concentrations of ^{13}C did not return at the asymptotic background concentration of the excretion curve. If an increased proportion of lignin in combination with

the findings of Benner *et al.* (1987) would hold, a stronger decline in the excretion line would be expected. However, in our herbages the differences between the naturally occurring levels of enrichment in lignin and those of the other fractions were only minor. Furthermore, the quantities of pulse dosed enriched materials were small compared to the pool sizes, making it unlikely that a hypothetical shift in lignin proportion would significantly affect the excretion patterns. In literature, *in situ* incubations of enriched material done by Svejcar *et al.* (1993) showed no discrepancy between the disappearance of ^{13}C and that of the DM. This proved that a change in the amount of ^{13}C gave no indications for an increase in the proportion of lignin in the residue. Therefore, in conclusion we assume that the internal marked fractions of ^{13}C that have been used in the current project, do represent the *in vivo* situation.

Use of ^{13}C as a marker in nutrition research. Based on the first above mentioned concept to alter the level of ^{13}C-enrichment in the nutrient pool, several groups have reported on methods to artificially enrich plants under laboratory and field conditions (Boutton *et al.*, 1987; Svejcar *et al.*, 1990; Svejcar *et al.*, 1993). Svejcar *et al.* (1993) enriched alfalfa under field conditions and subsequently fed the material to sheep. They were able to find traces of the ^{13}C back in faeces, blood serum and breath samples of animals. Based on the second concept (C_3 vs. C_4), Südekum *et al.* (1995) exchanged C_3-species (barley silage) based diets of steers for one day with a C_4-species (maize silage) and were able to establish faecal excretion patterns of ^{13}C. Both studies clearly showed the potential of ^{13}C to be used as an internal marker to study passage dynamics of feedstuffs through the animal's intestinal tract.
With regard to the current project, it was decided to study the passage dynamics of feed particles using artificially enriched materials. In Chapter 2 detailed information is given on the outcome of several enrichment trials with perennial rye grass (*Lolium perenne* L.) under field conditions. The main objective here was to develop and validate an enrichment procedure under field conditions that generated adequate amounts of enriched grass, which subsequently could be used in animal trials with dairy cows.

Assumptions and hypotheses. The over-all assumption in the current study was that the fermentative and digestive processes along the GI-tract show no isotopic discrimination between ^{12}C and ^{13}C isotopes. Because the ^{13}C is taken up by and incorporated into the different plant fractions through the naturally occurring process of assimilation, up to the moment of harvest the enriched materials should be identical to the untreated herbage. Therefore, it was assumed that information on fractional passage rates obtained from ^{13}C-marked fractions should be more representative for the *in vivo* situation. In combination with information that fractional passage rates do vary four main hypotheses were formulated that are summarised below (see also Chapter 1):

1. The passage of internally marked ^{13}C-feed fractions differ from the passage of traditionally used external markers.
2. Different feeds and feed components show differences in passage behaviour.
3. The passage of feeds and feed components are affected by factors like level of feed intake, diet composition and diet quality.

4. The rate of passage of feed particles from the rumen is largely related to the rate of degradation in the rumen.

In addition to these main hypotheses, the importance of several other issues is acknowledged. The choice of mathematical models describing the marker excretion patterns will ultimately determine the height of the rate constants. Moreover, the choice of model determines the compartmentalisation of the animal's gastro-intestinal tract and which processes are considered or disregarded (see Chapter 6). To address these issues and to

Table 1. Summary of animal, roughage and some diet characteristics, the fractional passage rates of markers afterintroduction in the rumen and the *in situ* determined fractional degradation rates.[1,2]

Chapter	3		4	5	
Roughage type	Grass silage		Fresh grass	Grass silage	
Dietary treatment	HI	LI	FG	GSH	GSL
Animal characteristics					
Body weight, (kg)	484	501	513	561	564
Days in milk, (d)	57	D[5]	239	136	145
Milk, (kg)	19.7	-	23.0	21.1	17.2
Fat, (%)	5.17	-	4.42	4.78	5.48
Protein, (%)	2.87	-	3.09	3.07	3.09
Dietary characteristics					
Daily intake, (kg DM/d)	12.5	7.6	16.8	16.5	15.2
Proportion Comp., (%)	41.6	42.7	21.4	45.0	47.3
Roughage characteristics					
DM, (g/kg product)	391.0	391.0	132.0	341	450.0
NDF, (g/kg DM)[3]	466.5	466.5	512.8	408.9	511.6
ADF, (g/kg DM)[3]	281.0	281.0	302.3	258.4	300.9
ADL, (g/kg DM)[3]	25.5	25.5	27.3	20.6	30.2
Fractional passage rates (K_1)[4]					
Co	12.95	11.11	13.98	13.16	10.61
Cr	6.88	4.66	4.31	5.34	5.34
^{13}CDM	3.33	2.61	2.49	4.27	3.42
^{13}CNDR	2.90	1.86	2.17	1.96	1.70
^{13}CNDS	3.90	3.02	3.05	4.71	4.06
Fractional degradation rates (K_d)[4]					
DM	2.85	2.85	2.75	3.20	2.60
OM	2.83	2.83	2.77	3.20	2.60
NDF	2.59	2.59	2.37	3.00	2.60
N	-	-	4.98	4.70	2.90

[1] HI = high intake level (Exp1, Chapter 3); LI = low intake level (Exp2, Chapter 3); FG = fresh grass; GSL = grass silage of lower digestibility; GSH = grass silage of higher digestibility.
[2] Data presented in the Table are arithmetic mean values within dietary treatment groups.
[3] Based on wet chemical analyses.
[4] Units in %/h.
[5] D = non-lactation animals.

answer the fourth hypothesis, data of individual experiments have been combined. The arithmetic mean values of some animal and feed characteristics and the fractional rates of passage (K_1) and *in situ* degradation (K_d) are summarised in Table 1. More detailed information on variations and differences between mean values can be obtained from the corresponding chapters. The values for K_1 represent the fractional rate of passage from the rumen as derived from faecal marker excretion curves.

The fractional passage rates show clear differences between the external and internal markers. Co-EDTA (Co) had the highest passage rates, which is in line with expectations. In all cases, the internal marker ^{13}C gave lower fractional rate constants than the external marker Cr-NDF (Cr), where passage rates of the cell wall fractions ($^{13}CNDR$) proved to be lower than those of the DM (^{13}CDM) and the non-cell wall fractions ($^{13}CNDS$). Animals at a higher level of feed intake (dietary treatment HI, Table 1) had higher K_1-values for the particle phase markers (Cr and ^{13}C-marked fractions) than animals at a lower level of feed intake. The K_1-values of Co and ^{13}C-marked fractions observed in GSH-fed animals tended to higher values compared to those in GSL-fed animals. The passage rate of Cr was not affected by this contrast in roughage type. These data (see also Chapter 5) suggest that ^{13}C has the capacity to distinguish between differences in passage rates as influenced by roughage quality as opposed to Cr. In addition, the silage of higher digestibility (GSH) gave higher fractional degradation rates. In combination with the differences in K_1 between treatments the data show the potential of ^{13}C to assess the relation between passage and degradation.

Relation Between Passage and Digestion

Passage of feed particles out of the rumen is assumed to depend on an appropriate functional specific weight of feed particles, which depends among others on fermentation gases entrapped in feed particles. This entrapment depends on (rate of) degradation. Hence, a relationship can be expected between rate of degradation and rate of passage. To assess the relationships between the fractional passage and degradation rates, only four measurement points (treatment groups) were included for each combination of K_1 (five markers) and K_d (four fractions). The degradation characteristics of the grass silage fed in the experiments described in Chapter 3 (Table 1) were determined *in situ* in animals kept under similar dietary conditions as the HI-treatment group. Therefore, data belonging to the dietary treatment group LI (Table 1) were excluded. In the same *in situ* trial the degradation characteristics of the N-fraction were not determined, hence, reducing the number of measurement points to three. The linear relations between fractional degradation and passage rates are summarised in Table 2 and graphically presented in Figure 1.

Linear regressions between the passage rates of Cr and the fractional degradation rates of the DM, OM and NDF gave low determination coefficients ($R^2 \leq 0.057$), indicating no

Table 2. Relations between the fractional passage rates of markers (Co, Cr, ^{13}CDM, ^{13}CNDR, ^{13}CNDS) and the fractional degradation rates of different fractions (DM, OM, NDF, N) of roughages.[1]

	Co	Cr	^{13}CDM	^{13}CNDR	^{13}CNDS	Co	Cr	^{13}CDM	^{13}CNDR	^{13}CNDS
	DM-fraction					OM-fraction				
μ	0.045	0.040	-0.024	0.015	-0.009	0.041	0.047	-0.022	0.017	-0.007
(SE)	0.099	0.083	0.041	0.040	0.042	0.098	0.085	0.043	0.041	0.044
α	2.857	0.523	2.015	0.233	1.692	3.012	0.264	1.961	0.161	1.632
(SE)	3.473	2.917	1.439	1.414	1.471	3.446	2.959	1.498	1.431	1.524
R^2	0.253	0.016	0.495	0.013	0.398	0.276	0.004	0.461	0.006	0.364
	NDF-fraction					N-fraction				
μ	0.141	0.029	-0.038	0.034	-0.027	0.061	0.063	0.039	0.011	0.047
(SE)	0.103	0.074	0.010	0.036	0.014	0.007	0.018	0.034	0.003	0.031
α	-0.523	0.961	2.729*	-0.442	2.495*	1.550†	-0.316	-0.116	0.197	-0.180
(SE)	3.885	2.774	0.383	1.348	0.530	0.172	0.417	0.783	0.067	0.719
R^2	0.009	0.057	0.962	0.051	0.917	0.988	0.365	0.022	0.897	0.059

[1] Regression equation: K_1(marker) = μ + α × K_d(fraction), where μ = intercept and α = regression coefficient; SE = standard error; R^2 = coefficient of determination.

[2] Number of experimental units regarding the fractions DM, OM, NDF (n=4), and N (n=3).

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

relationships. With respect to the N-fraction a non-significant and slightly negative relation was observed ($R^2 = 0.365$; $P = 0.587$). The relation between the ruminal passage rate (K_1) of Co and the degradation rate (K_d) of N gave a high determination coefficient ($R^2 = 0.988$), but, with only three observations included the effect was non-significant ($P = 0.070$). The relation between the K_1 of ^{13}CNDR and the K_d of N showed a similar relation ($P = 0.208$). The higher R^2-values observed in the relations between the K_1 of ^{13}CDM and ^{13}CNDS and the K_d of the DM ($R^2 \geq 0.398$; $P \geq 0.296$), OM ($R^2 \geq 0.364$; $P \geq 0.321$) and NDF ($R^2 \geq 0.917$; $P \leq 0.042$), suggest that these internal markers have a higher ability to quantify the relations between passage and degradation compared to the external markers. However, with regard to the K_d of the N-fraction no relations were observed. No linear relations were observed between the K_1 of ^{13}CNDR and the K_d of the DM, OM, and NDF-fractions.

Possible factors that contribute to the high R^2-value in the relation between the passage of the liquid phase (Co) and the degradation of N could be the different appearances in which protein occurs in plant tissues. An important part of the feed protein is readily soluble (Mangan 1982) and its passage behaviour will most likely be similar to that of the liquid phase. However, this part of the protein reflects the washable fraction and therefore, will have no direct influence on the relation between K_1 and K_d, as the latter is solely related to the insoluble but potentially degradable protein fraction. The insoluble part consists of a variety of protein complexes with variable and unknown degradation rates. Depending on the type of

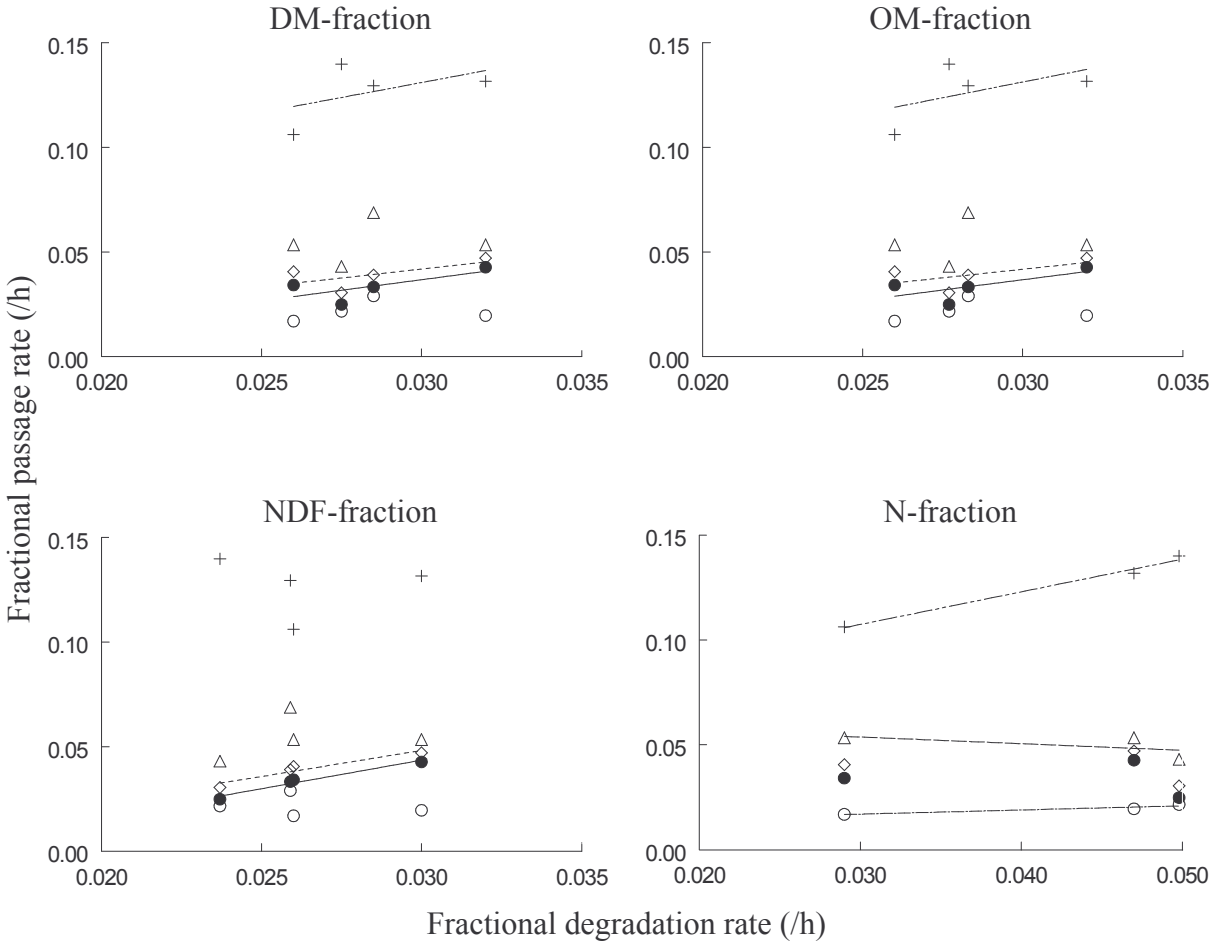

Figure 1. Relation between the fractional degradation rates of the DM, OM, NDF and N-fractions of different roughages and the observed fractional passage rates of different markers (Co-EDTA, −··−+−··−; Cr-NDF, −−−Δ−−−; ^{13}CDM, ——●——; ^{13}CNDR, −·−o−·−; ^{13}CNDS, -----◊-----). Only regression lines with an $R^2 > 0.25$ are presented.

roughage used in the animal experiments, a change in the proportion of these insoluble but more easily degradable proteins might be one of the factors contributing to the observed relation between Co passage and N degradation. Another part of this insoluble protein fraction (*extensin*) is associated with the cellulose complexes in the primary cell walls (Fry 1988). For this protein fraction it may be assumed that its passage behaviour will largely follow that of the cell walls, which would explain the high correlation between the passage of ^{13}CNDR and degradation of N. Considering the above, it was in line with the expectation that the degradation of the 'third' or rather remaining part of the insoluble protein fraction would to some extent be related to the passage of ^{13}CDM or ^{13}CNDS. However, no relations were observed here although these markers showed promising relationships with de degradation rates of the DM and OM-fractions.

Regarding the positive and significant relations between the K_1 of ^{13}CDM and ^{13}CNDS and the K_d of NDF, the slightly negative but non-significant relation between the K_1 of ^{13}CNDR and the K_d of NDF was somewhat unexpected. As the latter relation concerns information on the passage and degradation of cell wall specific components, it was expected to show a more

distinct relationship between K_1 and K_d. However, data in Table 1 show that the range of the values for both K_1 (^{13}CNDR; min. 0.0170/h, max. 0.0290/h) and K_d (NDF, min. 0.0237/h, max. 0.0300/h) were very small. In addition to the other sources of variability, these narrow ranges form an extra impediment in determining relationships. In conclusion, our results showed that fractional passage rates of ^{13}CDM and ^{13}CNDS were in general positively related to the degradation of the DM, OM and NDF, opposed to the passage of cell walls (^{13}CNDR), which showed no clear relationships with these fractions. The external particle phase marker (Cr) showed no relation between passage and degradation and appears to be an inappropriate marker to be used in studies to relate passage of feed particles to the degradation of feed particles components. However, the relations described here are based on a limited number of data obtained from considerably contrasting animal trials and hence, they should be considered with care. With respect to answering the hypothesis '*passage is related to degradation*', our observations show the potential of ^{13}C as an internal marker to assess the relation between passage and degradation.

Implications for Feed Evaluation

The Dutch protein evaluation system. One of the issues that formed the basis of the current study was that within the Dutch protein evaluation system (DVE-system) the amount of rumen undegradable feed protein (BRE) is determined by assuming fixed values for the fractional passage of feedstuffs. Therefore, the major questions to answer are, if and/ or how these results could be integrated in the current DVE-system and what implications they would have on the nutritional status of the dairy cow. Our observations (Chapters 3, 4, and 5) clearly showed that factors like level of feed intake, type and quality of roughage affect passage, which is in line with literature (Tamminga *et al.*, 1989; Colucci *et al.*, 1990; Bruining and Bosch, 1991; Mambrini and Peyrauld, 1994; Rinne *et al.*, 1997; Lund, 2000). The fractional passage rate constants adopted in the current DVE-system are weighed averages based on work where different types of external markers have been used (Verité *et al.*, 1987; CVB, 1991). The use of ^{13}C as an internal marker made it possible to acquire knowledge on the feed and feed component specific passage behaviour of feed particles. These results were evaluated against the passage of traditionally used external markers Co-EDTA (liquid) and Cr-NDF (particle). In all cases the ^{13}C-enriched fractions (DM, NDR and NDS) gave lower passage rates compared to Cr-NDF (see also Table 1). In addition, results showed that passage rates between enriched fractions also differed considerably. In general, results suggested that Cr-NDF overestimates the fractional passage from the rumen and a first conclusion would be that the fixed values currently used in the DVE-system overestimate the *in vivo* situation, and hence, overestimate the amount of rumen undegradable protein. However, the ^{13}C-enriched fractions used in our trials will to a large extent represent the lesser degradable structural carbohydrate fractions of the roughage. Therefore, direct integration of these observed feed

specific fractional passage rates into a system that evaluates the protein status have to be done with careful attention. It should be noted that, in addition to the effect of fractional passage rate on rumen bypass protein, fractional passage rate is also a major determinant of microbial protein efficiency (Dijkstra *et al.* 1998). However, microbial efficiency in the Dutch DVE system is fixed at 150 g microbial protein per kg FOM independent of dietary situation and will not be considered further in this discussion

Quantifying the effect of introducing feed specific fractional passage rates. To calculate the amount of fermentable organic matter (FOM) the apparent (faecal) digestible organic matter (DOM) is corrected for the amount of rumen undegradable protein (BRE), the amount of rumen undegradable starch (BZET), the amount of crude fat and the fermentation end products resulting from the ensiling process, where the amounts of BRE and BZET are functions of the fractional passage (Tamminga *et al.*, 1994). By introducing the lower feed specific fractional passage rates, the amounts of BRE and BRE available for absorption in the small intestine (DVBE) will decrease, which results in a higher FOM. The decrease in DVBE will to some extent be compensated by an increase in the amount of synthesised microbial protein that enters the duodenum (DVME). However, since the DVME is calculated as a fixed proportion of the FOM (± 10% of FOM; 0.15×0.75×0.85) this effect is only minor. The equations underlying the DVE-system are described in detail by Tamminga *et al.* (1994). The influence of introducing feed specific fractional passage rates into the DVE-system on the height of some parameter estimates within this system is summarised for three roughages (Table 3). These data clearly show that the extent of the effect (i.e., the change in DVE due to change in K_1) depends on the type and quality of the roughage. Values obtained by using K_1-values of Cr-NDF match the estimations that are based on the assumed fixed passage rates for roughages of 0.045/h. However, with the internal markers in general the amount of protein available for digestion in the small intestine (DVE) will decrease, whilst the values for the degradable protein balance (OEB) increase. The effects on DVE and OEB were more pronounced in fresh grass (FG) compared to the two grass silages (GSH, GSL), which is explained by the lower K_1-values for ^{13}CDM and ^{13}CNDS in the FG-group. The relatively high amounts of BRE and DVBE in fresh grass are likely related to the late stage of maturity at which FG was harvested.

In conclusion our observations indicate that the adopted fixed value for the fractional passage of forages in the current system overestimates the amounts of DVBE and DVE, and underestimates the FOM and the OEB. Introduction of feed (component) specific fractional passage rates seems to offer perspective to more accurately estimate the feeding values of feeds and hence, give more discriminating information on the nutritional status of the animal.

A DVE-system based on variable fractional passage rates. After the preceding discussion the question remains how these variable fractional passage rates could be integrated in the current DVE-system. One approach would be to adjust the current fixed value of 0.045/h for roughages to a lower but still arbitrarily fixed value. However, such an approach would disregard the acquired knowledge on differences in passage rates between feed components, as affected by

Table 3. The influence of variable fractional passage rates on some parameter estimations within the DVE-system.[1,2]

	K_1 (/h)	%BRE (%)	BRE[3]	%DVBE (%)	DVBE[3]	FOM[3]	DVME[3]	DVMFE[3]	DVE[3]	OEB[3]
*Fresh grass (Dietary treatment **FG**; Table 1)*										
CVB	0.0450	35.4	67.7	79.4	53.7	656.0	62.7	17.7	98.8	5.9
Cr-NDF	0.0431	34.8	66.5	79.0	52.6	657.1	62.8	17.7	97.7	7.0
^{13}CDM	0.0249	27.1	51.7	73.0	37.7	670.4	64.1	17.7	84.2	19.8
^{13}CNDR	0.0217	25.3	48.3	71.1	34.3	673.5	64.4	17.7	81.1	22.7
^{13}CNDS	0.0305	29.8	56.9	75.5	43.0	665.7	63.7	17.7	89.0	15.2
*Grass silage of low digestibility (Dietary treatment **GSL**; Table 1)*										
CVB	0.0450	38.3	53.2	56.7	30.1	606.5	58.0	21.4	66.8	-19.1
Cr-NDF	0.0534	39.7	55.1	58.2	32.1	604.7	57.8	21.4	68.6	-20.8
^{13}CDM	0.0342	35.9	49.8	53.8	26.8	609.5	58.3	21.4	63.7	-16.3
^{13}CNDR	0.0170	29.8	41.3	44.3	18.3	617.2	59.0	21.4	55.9	-8.9
^{13}CNDS	0.0406	37.4	51.9	55.6	28.9	607.6	58.1	21.4	65.6	-18.1
*Grass silage of high digestibility (Dietary treatment **GSH**; Table 1)*										
CVB	0.0450	24.1	52.9	71.7	37.9	682.8	65.3	16.0	87.2	42.7
Cr-NDF	0.0534	25.6	56.2	73.4	41.2	679.8	65.0	16.0	90.2	39.8
^{13}CDM	0.0427	23.6	51.9	71.2	36.9	683.7	65.4	16.0	86.3	43.6
^{13}CNDR	0.0196	17.2	37.8	60.5	22.8	696.4	66.6	16.0	73.4	55.7
^{13}CNDS	0.0471	24.5	53.8	72.2	38.8	682.0	65.2	16.0	88.0	41.9

[1] The respective values for the washable (**W**) and undigestible (**U**) fractions and the degradation rates (K_d) for treatment FG: 33.4%, 7.3%, 0.0498/h; GSL: 47.7%, 16.6%, 0.0290/h; GSH: 58.0%, 6.8%, 0.0470/h.

[2] K_1 = fractional passage rate from the rumen; %BRE = U+(100-W-U)*(K_1/K_1+K_d) ; BRE =amount of undegradable protein; %DVBE = % of undegraded feed protein available for digestion; DVBE = amount of undegraded feed protein available for digestion; FOM = fermentable organic matter; DVME = amount of microbial protein available for digestion; DVMFE = amount of endogenous protein in the small intestine; DVE = amount of protein available for digestion in the small intestine; OEB = undegraded protein balance.

[3] Amounts in g/kg DM unless mentioned otherwise.

some dietary and animal factors. Therefore, another approach could be to adopt variable rate constants for passage in the evaluation system. The amount of undegradable protein (BRE) could then be estimated from a pre-set range of passage rates where a combination of factors ultimately determines the height of the rate constant. Factors to be included will be proposed below, but the discussion will primarily centre on the roughage component of the diet as no information on concentrate components was collected within the current project.

In analogy with the current DVE-system the available feed resources would be categorised into two groups; the roughages and the compound feeds. However, within a category (here roughages), fractional passage rate constants (K_1) of which the height depends on the type and quality of the roughage could be used. To further improve the estimation on feed protein values, it would be advisable to a further differentiation between the protein fractions. Information

obtained from *in situ* experiments form the basis of our protein evaluation system that currently acknowledges a soluble (S) or rather washable part (W), a truly undigestible part (U) and an insoluble potentially degradable part (D). The discrepancy between the soluble and the washable part has been recognised (Goelema, 1999), and in ongoing efforts the relations are studied. Currently, the D-fraction of the feed protein is regarded as one entity, whilst in reality it consists of a variety of protein complexes. This would consist of a more easily degradable part and the part associated with the primary cell wall constituents (*extensin*). Their proportion would be a function of several factors like cell wall composition, harvest date and/ or length of the regrowth period. In summary, the S-fraction of protein that potentially escapes from the rumen would be described as to depend on the fractional passage rate of the liquid phase. The washable but insoluble fraction is to date poorly characterised, which is also true for the knowledge on its passage behaviour. However, it is likely to assume that the passage of this fraction will correspond with the liquid phase as well. The fractional passage of the more difficult degradable fraction *extensin* could be described by that of the cell walls (^{13}CNDR), whilst the remaining protein in the D-fraction could be associated with either the passage of ^{13}CDM (DM) or ^{13}CNDS (non-cell walls). The current study showed that the stable isotope of carbon forms a promising tool to make a distinction between the passage rates of feed components under different dietary conditions.

A combination of animal related factors (e.g. body weight, milk production, genotype) and dietary factors (e.g. roughage type and quality, diet composition and quality, amount of concentrate in the diet) leads ultimately to a certain level of feed intake and an amount of milk produced. Therefore, it is fair to assume that an interaction of animal and dietary factors will also determine the height of the fractional passage rates. In Chapter 6 an onset was made to relate the compartmental retention time (reciprocal of K_1) to a combination of animal and dietary factors that are available on-farm. The equations developed from these relations, combined with easily accessible on-farm information, allows to generate feed (component) specific fractional passage rates, which are corrected for a specific on-farm situation (tailor made passage rate). Such an equation may be compared to the approach adopted in the UK metabolisable protein system, where an empirical function is used to define rumen outflow rate in relation to feed intake level. Calculated outflow rate in this system varies from 0.019 to 0.104/h at maintenance and 4.5 times maintenance level, respectively (AFRC, 1992).

In conclusion, it is envisaged that a combination of the factors discussed above will result in a matrix, where the height of K_1 is a function of farm, group, animal, feed and feed component specific criteria. Figure 2 shows a simplified three dimensional illustration of the variability in fractional passage rate for ^{13}CDM, as affected by the total daily DM intake and body weight. The values for K_1 have been obtained by calculating the reciprocal from the equation;

$$\text{CMRT1}(^{13}\text{CDM}) = -452.5 + 0.212 \times \text{BW} - 4.4 \times \text{DMI} - 2.471 \times \text{NDF} + 5.69 \times \text{ADF} \qquad [\,1\,]$$

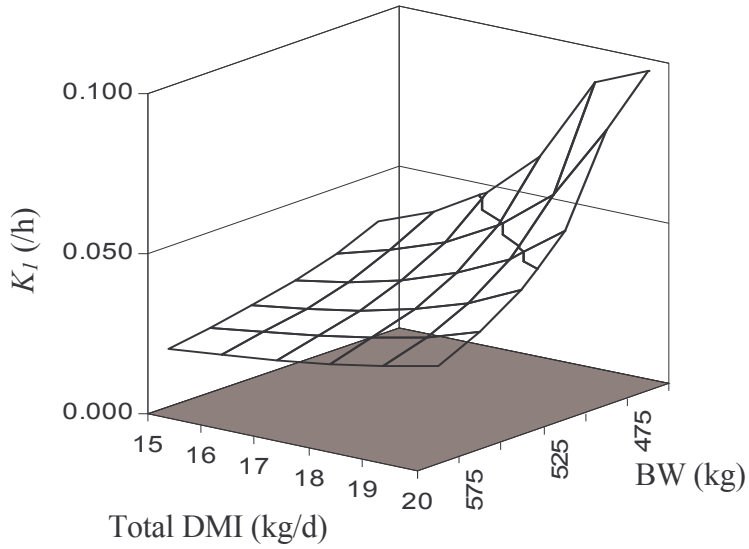

Figure 2. Relation between the total daily DM intake (DMI, kg/d) and body weight (BW, kg) and its effect on the fractional passage rate (K_1). K_1-values are based on the equation describing the CMRT1 for marker ^{13}CDM (see Table 6, Chapter 6).

For details see Table 6, Chapter 6. Because our data is based on a limited number of experimental conditions, it would require some extensive validation before extrapolation would be possible. Figure 2 clearly demonstrates that extrapolation to higher levels of DMI in combination with lower body weights result in unrealistic fractional passage rates.

From DVE to NB (nutrient based) system. The previous discussion shows that a proper implementation of feed specific fractional passage rates into the DVE-system will demand a considerable input of research capacity, as knowledge on this topic is limited and certainly not sufficient. However, it remains questionable whether it is still worthwhile to allocate large sums of research budgets and manpower into the development of this system, especially when considering that it contains several major limitations. Important constraints are; (i) no integration between protein and energy metabolism; (ii) lacking in information regarding nutrient type (glucogenic, aminogenic and lipogenic) and the partitioning of these nutrients into the animal; (iii) lacking in predicting the animals response to dietary changes (AFRC, 1998; Tamminga *et al.*, 2000).

Future developments in feed evaluation show preferences towards systems of a more mechanistic, dynamic nature, where the energy and protein supply to the animal are fully integrated entities. Tamminga *et al.* (2000) presented a proposal for such a nutrient-based feed evaluation system for ruminants. The model would include 11 modules that simulate the digestive and metabolic processes in the animal. This collection of modules would operate under an input/ output interface. Through the input interface dietary, animal and environmental factors are to be introduced in the modules. Via the output interface information can be obtained on milk production and composition, faecal and urinary output, etc. A schematic representation of the proposed model is given in Figure 3. The simulation

model compartmentalises the GI-tract into a rumen, small intestine and large intestine compartment. To accurately describe the fate of the individual nutrients in the successive compartments, knowledge on feed (component) specific passage rates become essential. With the proposed input interface by the model of Tamminga *et al.* (2000) it would be possible to directly implement feed specific passage rates as measured and described in this thesis.

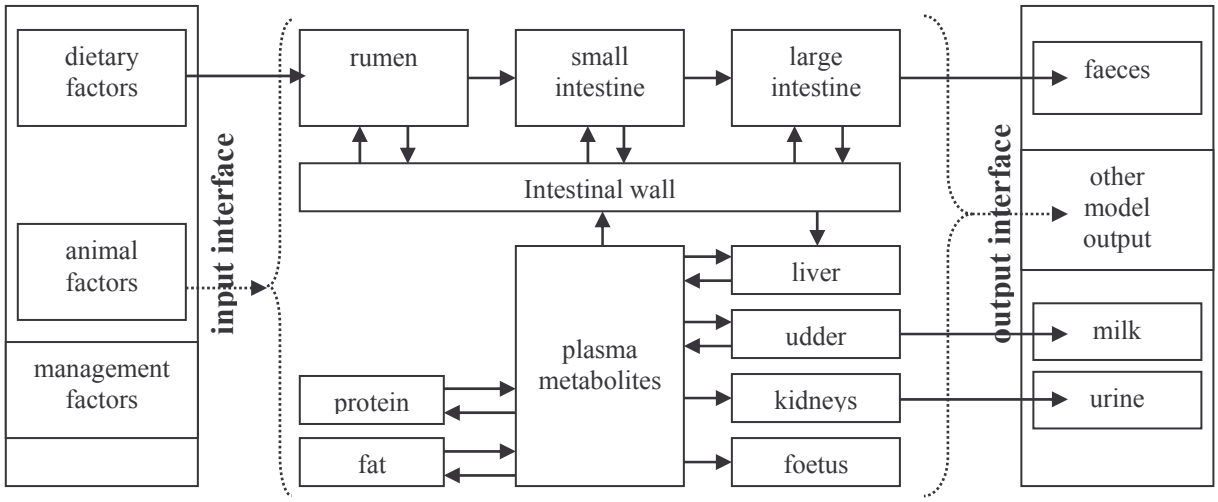

Figure 3. A schematic representation of a nutrient based feed evaluation system, adopted from Tamminga *et al.* (2000).

General Conclusions

The main conclusions that can be drawn from the work presented in this thesis are:

- Marker excretion patterns of ^{13}C in the dry matter (DM), the cell wall (NDR) and the non-cell wall (NDS) fractions are well fitted by non linear stochastic and deterministic models. The observed standard errors of the mean parameter estimates (SEM), the coefficients of determination (R^2), and other curve fit evaluation methods like the mean prediction error (MPE) do not differ from those obtained with the traditionally used external markers (Co-EDTA, Cr-NDF). Therefore, ^{13}C forms a reliable internal marker that generates accurate information on the passage dynamics of feed fractions.

- Compared to the two-compartment stochastic models described by Pond *et al.* (1988), the multi-compartmental model (MC-model) as described by Dhanoa *et al.* (1985) proved to be superior in fitting excretion curves.

- In future experimentation it is recommended to increase the number of experimental units (more animals) or the experimental degrees of freedom (repeated measures). In the current experiments variation in fractional passage rate estimates was often rather large and the number of measurements low, hence, lowering the chance to obtain significant differences ($P < 0.05$) between treatments.

- Passage rates of the ^{13}C-enriched fractions differed clearly from those measured with the external particle phase marker (Cr), which in turn differed from the passage of the liquid phase marker (Co).

- In all cases Co gave significant higher fractional passage rates compared to the other markers (Cr, and ^{13}C in the DM, NDR and NDS), which was in line with the expectation.

- In most situations, the internal markers had distinctly lower fractional passage rates than Cr, with the largest contrasts between Cr and $^{13}CNDR$.

- The passage dynamics of the DM, NDR and NDS fractions were considerably different. The compartmental (CMRT's) and total residence times (TMRT's) of the cell wall fraction ($^{13}CNDR$) following from rumen pulse doses, were significantly longer than those of ^{13}CDM and $^{13}CNDS$. In analogy, the moment when the highest marker concentration was reached (PCT) as well as the length of the transit time (TT) were both at a considerable later stage for $^{13}CNDR$ compared to ^{13}C in de DM and NDS.

- The passage behaviour of all markers was affected by the level of feed intake. In case animals were subjected to moderate levels of feed restriction, the residence time in all gastro-intestinal compartments increased. As a result, the feed components will be subjected to fermentation for a longer time, which eventually determines how, where and when nutrients will become available to the animal.

- Grass silage of higher digestibility (GSH) tended to shorter residence times (higher fractional passage rates) then silage of lower digestibility (GSL). The ^{13}C-enriched fractions were able to detect differences in passage rates between silage treatments as well as between different fractions.

- The use of ^{13}C as an internal marker allows differentiating between the passage behaviour of different feed components. Both chemically and mathematically ^{13}C is well traceable through the GI-tract of animals. Moreover, ^{13}C-enriched feed stuffs are subjected to the normally occurring ruminating and fermenting processes, and hence, it may be concluded that for the *in vivo* situation ^{13}C is more representative than the external markers.

- ^{13}CDM and ^{13}CNDS gave promising results in quantifying the relations between fractional passage rates and the *in situ* fractional degradation rates. In contrast, the passage of the cell wall fraction (^{13}CNDR) was poorly related to the degradation rates of the different fractions (DM, OM, NDF and N). However, the number of data points was small and showed little variation and hence, our observed relations should be considered cautiously. Nevertheless, the results suggest that ^{13}C is a promising tool to quantify the relation between passage and degradation.

Literature Cited

AFRC. 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. AFRC Technical Committee on Responses to Nutrients. Report no. 11. Nutr. Abstr. Rev. (Ser. B) 68: 937-989.

AFRC. 1992. AFRC Technical Committee on responses to nutrients, Report number 9, Nutritive requirements of ruminant animals: protein. Nutr. Abstr. Rev. (Ser. B) 62: 787-835.

Benner R., M. L. Fogel, E. Kent Sprague and R. E. Hodson. 1987. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. Nature. 329:708-710.

Bosch, M. W. and M. Bruining. 1995. Passage rate and total clearance rate from the rumen of cows fed on grass silages differing in cell-wall content. Brit. J. Nutr. 73(1):41-49.

Boutton, T. W. 1991. Uses and procedures for ^{13}C. *in*: Carbon isotope techniques. D.C. Coleman and B. Fry, eds. Academic Press Limited, London, UK:155-186.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, I. McMillan, and D. J. Barney. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 73(8):2143-2156.

CVB, Centraal Veevoeder Bureau. 1991. Eiwitwaardering voor herkauwers: het DVE-systeem. CVB-reeks nr. 7, ISSN nr. 0925-546X.

Dijkstra, J., J. France and D. R. Davies. 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. J. Dairy Sci. 81, 3370-3384

Ehleringer, J. R. 1991. Uses and procedures for ^{13}C. *in*: Carbon isotope techniques. D.C. Coleman and B. Fry, eds. Academic Press Limited, London, UK:187-200.

Fry, S.C. 1988. The growing plant cell wall: chemical and metabolic analysis. Longman Scientific & Tchnical, Harlow, UK.

Goelema , J. O., B. H. G. Boswerger, I. van Deurzen and G. Hof. 1999. Effect of various conditions during pressure toasting on the rumen degradability and intestinal degradability of faba beans, peas, lupins and soybeans. In: Ph.D. Diss., Wageningen Agricultural University, The Netherlands.

Kimball, J. D. 2004. Kimball's biology pages. http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/W/Welcome.html. accessed 11th March 2004.

Lund, P. 2002. The effect of forage type on passage kinetics and digestibility of fibre in dairy cows. The Royal Veterinary and Agricultural University, Copenhagen.

Mambrini, M. and J. L. Peyraud. 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with a maize silage diet. Reprod. Nutr. Dev.. 34(1):9-23.

Mangan, J. L. 1982. The nitrogenous constituents of fresh forages. In: D. J. Thomson, D. E. Beever and R. G. Gunn (eds.) Forage protein in ruminant animal production No. 6. p 25-40. Occasional Publication, British Society of Animal Production.

O'Leary, M. H. 1988. Carbon isotopes in photosynthesis. BioScience. 38:328-336.

Rinne, M., P. Huhtanen, and S. Jaakkola. 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol. 67(1):19-35.

Rodrigues, S. I. F. C., J. Dijkstra, A. G. Assis, J. France, and S. Tamminga. 2002. Evaluation of supplements to enhance nutrient supply and milk production of cattle fed diets based on elephant grass (Pennisetum purpureum) using a mathemetical model. Livest. Prod. Sci. 77:273-286.

Smith, B. N. and S. Epstein. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiology. 47:380-384.

Südekum, K. H., W. Ziggers, N. Roos, H. Sick, S. Tamminga, and M. Stangassinger. 1995. Estimating the passage of digesta in steers and wethers using the ratio of ^{13}C to ^{12}C and titanium(IV)-oxide. Isotopes Environ. Health Stud. 31:219-227.

Svejcar, T. J., T. W. Boutton and J.D. Trent. 1990. Assessment of carbon allocation with stable carbon isotope labeling. Agronomy Journal. 82:18-21.

Svejcar, T. J., M. B. Judkins, and T. W. Boutton. 1993. Technical note: Labeling of forages with ^{13}C for nutrition and metabolism studies. J. Anim. Sci. 71(2):1320-1325.

Tamminga, S., W. J. J. Gerrits, A. Bannink, J. Dijkstra, G. A. L. Meijer, and A. M. van Vuuren. 2000. Een opzet voor een nieuw, op nutriënten gebaseerd voederwaarderingssysteem voor herkauwers. 25ste studiedag voor Nederlandstalige voedingsonderzoekers, Wageningen:20-21.

Tamminga, S., P. H. Robinson, M. Vogt, and H. Boer. 1989. Rumen ingesta kinetics of cell wall components in dairy cows. Anim. Feed Sci. Technol. 25(1-2):89-98.

Tamminga, S., W. M. van Straalen, A. P. J. Subnel, R. G. M. Meijer, A. Steg, C. J. G. Wever, and M. C. Blok. 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40(2):139-155.

Vérité, R., B. Michalet-Doreau, P. Chapoutot, J. L. Peyraud, and C. Poncet. 1987. "Révision du systeme des protéines digestibles dans líntestin (P.D.I.)". Bulletin Technique C.R.Z.V. Theix, INRA. 70:19-34.

List of Abbreviations

At%^{13}C	atom percentage ^{13}C
δ^{13}C	delta ^{13}C vs. Pee Dee Belemnite
^{13}CDM	^{13}C in the DM
^{13}CNDR	^{13}C in the NDR
^{13}CNDS	^{13}C in the NDS
A	initial marker concentration at t = 0
ADF	acid detergent fiber
ADL	acid detergent lignin
ADLR	acid detergent lignin residu (ADL analyses but excluding the ashing step)
ADR	acid detergent residu (ADF analyses but excluding the ashing step)
APE	atom percentage excess
CMRT	compartmental mean residence time
Co	Co-EDTA
Cr	Cr-NDF
FT	feeding time
GSH	grass silage of high digestibility
GSL	grass silage of low digestibility
HI, LI	high intake, low intake
IR	intake rate
IRf	fractional intake rate
K, K_p	fractional passage rate constant derived from a single exponential equation
K_1	fractional rate of passage from the slowest compartment
K_2	fractional rate of passage from the second slowest compartment
K_c	fractional rumen clearance rate
K_d	fractional degradation rate
MPE	mean prediction error
N	number of compartments
NDF	neutral detergent fibre
NDR	neutral detergent residue (NDF analyses but excluding the ashing step)
NDS	neutral detergent solubles (DM - NDR)
OM	organic material
PCT	moment of peak concentration
RP	marker recovery percentage
SD	standard deviation
SEM	standard error of the mean
T0, T30, T90	moment of rumen liquid sampling relative to the moment of feeding, where 0, 30 and 90 refer to the time after roughage feeding in min.
TAF	time after feeding (T0, T30, T90)
TC	total carbon content
TMRT	total mean residence time
TT	time to first marker appearance (transit time)
VFA	volatile fatty acid

SUMMARY

Within the current Dutch feed evaluation system for ruminants the energy and protein supply to individual animals is being described by the VEM and DVE/OEB-systeem. The supply of protein is described as the sum of a bypass feed protein component (DVBE), a microbial component (DVME), a correction factor for endogenous losses (DVMFE) and the digestibility of these components in the small intestine. The amount of feed protein escaping from rumen fermentation depends on fractional degradation rates and fractional passage rates. The degradation rates are feed specific and are obtained by use of *in sacco* experiments (Tamminga *et al.*, 1994). With regard to the fractional passage rates, fixed values have been adopted in the DVE-system; 0.045/h for protein in roughages and 0.060/h for protein in concentrates (Vérité *et al.*, 1987; CVB, 1991). It is increasingly realised that also passage rates vary and depend amongst others on roughage type (Mambrini and Peyraud, 1994; Lund, 2002), roughage quality (Bruining en Bosch, 1991; Rinne *et al.*, 1997), diet composition and level of feed intake (Tamminga *et al.*, 1989; Colucci *et al.*, 1990). Therefore, it seems plausible that by introducing feed specific fractional passage rates the accuracy in estimating nutrient availability and nutrient utilisation can be improved.

In many studies on passage dynamics of feed components often external markers like Cr-NDF (particle phase) and Co-EDTA (liquid phase) are being used. In case of Cr-NDF the chromium is mordanted to the indigestible part of the cell wall fraction of straw (Udén *et al.*, 1980). After pulse dosing the marker in the rumen, fractional passage rates can be obtained from the decline in marker concentration with time determined in the rumen content, or in the chyme or faeces. Here it is assumed that the passage behaviour of the markers are representative for the feed particles originating from the diet. However, it is more likely that Cr-mordanted straw particles have a different passage behaviour than the feed particles, and hence, do not accurately describe the actual situation (Tamminga *et al.*, 1989). Therefore, to obtain information on feed specific fractional passage rates the use of internal markers are preferred as they are more representative for the *in vivo* situation.

Based on the aforementioned considerations, in the summer of 1997 a project was initiated to study the passage behaviour of feed particles and feed components through different compartments of the gastro-intestinal tract of dairy cows. To acquire information on feed specific fractional passage rates of different feedstuffs and feed components, the stable isotope of carbon (^{13}C) was used as an internal marker.

In **chapter 2** four labelling experiments are described where grass is being enriched under field conditions with ^{13}C. During the enrichment procedures ^{13}C has been incorporated notably in the cell wall fractions of plants through the natural process of assimilation. Depending on the objectives stated for the animal experiments, the enriched grass was either directly fozen (1x) or ensiled with the bulk silage in permeable nylon bags and thereafter frozen (3x). The enriched materials were analysed for cell wall composition (NDF, ADF and ADL), and by omitting the ashing step the respective residues (NDR, ADR and ADLR) were

obtained. In these residues the level of ^{13}C-enrichment was determined. The level of enrichment of the ADLR under natural conditions (unlabelled) was 1 to 3 $\delta^{13}C$-units lower than the other fractions, which agrees with literature. In the artificially enriched materials this effect became more pronounced, with significantly lower levels of enrichment in the ADLR compared to the other fractions. This was probably related to selection processes against ^{13}C that occur during the formation of biosynthetic precursors of lignin, phenylalanine and tyrosine.

The primary target of these trials was to develop a labelling procedure for perennial rye grass under Dutch field conditions that generate sufficient amounts of labelled material for use in passage studies in dairy cows. In all situations this target was reached.

Chapter 3 describes two animal trials in which the passage dynamics of the external markers Co-EDTA (Co) and Cr-NDF (Cr) are compared with that of ^{13}C as internal marker in the dry matter (^{13}CDM), the cell wall fraction ($^{13}CNDR$) and non-cell wall fraction ($^{13}CNDS$). After incorporating the ^{13}C into the plant fractions under field conditions the enriched grass was harvested and ensiled. Subsequently, the ^{13}C-enriched silage was introduced (pulse dosed) in the gastro-intestinal tract (GI-tract) of dairy cows, together with Cr-NDF and Co-EDTA. The rumen and ileum cannulated animals received a diet of grass silage (60%) and a specially designed compound feed (40%). During the first experiment, the animals were in early lactation and had a daily DM intake of 12.3 (sd = 0.3) kg (High Intake). In the second experiment the daily DMI was set at 60% of that in the first experiment (Low Intake). Animals received successive pulse doses in the ileum, abomasum and rumen. Subsequently, the faecal and ileal excretion patterns of Co, Cr, ^{13}CDM, $^{13}CNDR$ and $^{13}CNDS$ were determined. Upon ileal and abomasal pulse dosing, no significant differences between markers were observed. After ruminal pulse dosing, ^{13}CDM gave a distinctly lower fractional passage rate than the external markers. Comparison between the ^{13}C-marked fractions showed that $^{13}CNDR$ had the lowest fractional passage from the rumen however, differences in passage rates between the fractions were non-significant. A comparison between experiments (High vs. Low) clearly showed that a restriction in feed intake decreases the fractional passage rates of markers. The parameters generated by the non-linear models used for curve fitting show clear differences in passage behaviour of the DM, NDR and NDS-fractions. This indicates that nutrients become available in the GI-tract at different moments and in different quantities, and suggests that ^{13}C can be used to distinguish between fractions and/ or nutrients. Regarding the future development of nutrient based feed evaluation systems ^{13}C appears to be a potential tool to acquire information on feed and feed component specific fractional passage rates.

In **chapter 4** the passage dynamics of Co, Cr, ^{13}CDM, $^{13}CNDR$ and $^{13}CNDS$ are being studied in grass fed dairy cows. The rumen and ileum cannulated animals received a diet of fresh grass (75%) and a specially designed compound feed (25%). ^{13}C-labelled grass together with Co-EDTA and Cr-NDF were pulse dosed into the ileum, abomasum and rumen. Subsequently, the faecal and ileal excretion patterns of Co, Cr, ^{13}CDM, $^{13}CNDR$ and $^{13}CNDS$ were determined and fitted to non-linear models. The passage behaviour of the internal markers was distinctly different from the external markers, and within the internally marked fractions

clear differences were observed between the passage of DM, NDR and NDS. Upon ileal and abomasal pulse dosing, differences between markers were non-significant. However, after an abomasal pulse dose Co tended to higher passage rates. After pulse dosing in the rumen Co had a significantly higher fractional passage rate compared to the other markers. Here, Cr tended to higher passage rates compared to the ^{13}CDM and ^{13}CNDR. Within the ^{13}C-marked fractions ^{13}CNDR tended to lower passage rates than those of ^{13}CDM and ^{13}CNDS, resulting in considerable longer retention times in the GI-tract. Also these results suggest that nutrients become available in the GI-tract in different quantities at different moments, and at different sites. Besides, the results indicate that ^{13}C allows quantifying the difference in passage behaviour between different fractions, which is not possible when using external markers.

For the experiment described in **chapter 5**, three rumen and ileum cannulated dairy cows were used in a double cross-over design. At the onset animals were in early lactation and were randomly assigned to their dietary treatments; a grass silage of low digestibility (GSL) versus a grass silage of high digestibility (GSH). The animals received a diet of GSL or GSH (54%) and a specially designed compound feed (46%). The animals received successive pulse doses in the ileum, abomasum and rumen of Co-EDTA, Cr-NDF and one of the enriched silages, depending on their dietary treatment, and subsequently, the excretion patterns of Co, Cr, ^{13}CDM, ^{13}CNDR and ^{13}CNDS were determined. Feacally determined fractional passage rates upon ileal and abomasal pulse dosing showed no differences between markers, indicating that the contrast in silage digestibility did not measurably affect the passage of markers through the post-ruminal part of the GI-tract. After ruminal pulse dosing, the fractional passage rates for ^{13}CDM and ^{13}CNDR were considerably lower than those of Co and Cr. The combined information on the transit times, the moment when peak concentrations were reached, the compartmental and total mean residence times, indicated that ^{13}C-markers were more affected by the dietary treatment than the external markers. Therefore, it was concluded that ^{13}C is a more sensitive marker to distinguish differences in passage behaviour resulting from the dietary treatment compared to the external markers. Besides, in relation to ruminal degradation and clearance, more rational passage rates were obtained in case of the ^{13}C-marked fractions.

The mathematical models available to describe marker excretion patterns can be assigned to two different approached; a stochastic and a deterministic approach. In chapter 6 several stochastic models, varying in so-called gamma-time distribution (gamma-N, 1 to 5), are compared with the deterministic multi-compartmental models used in our studies. The choise of model approach is of considerable importance as it amongst others determines the researchers' interpretation of the excretion pattern. In case of stochastic models the influence of the caecum/ large intestine on the excretion pattern is assumed to be of minor influence, whilst in the deterministic model this segment is regarded to be the second slowest compartment.

Combining all excretion patterns of different markers and experiments resulted in a dataset large enough to test for differences between and the robustness of the various models. The deterministic model gave in general good results in fitting the excretion patterns. Stochastic models with lower orders of gamma-distributions (gamma-N < 3) gave in general poorer fits

than models with higher order gamma-distributions. The faecally determined excretion patterns of internal markers upon ileal and abomasal pulse doses gave relatively a lower number of false fits than those of Co and Cr. Faecally and ileally determined excretion patterns obtained after ruminal pulse doses showed no differences between markers. It was concluded that the model fits of the excretion patterns of the internal markers gave good results when compared to the curve fits of the external markers. The deterministic model gave good fits and appeared to be rather robust compared to the other models.

Based on the parameter estimates from the deterministic model, an attempt was made to relate the faecally determined compartmental residence times (upon ruminal pulse doses) to physical compartments, where the use of rumen and ileum cannulated animals allowed us to physically compartimentalise the GI-tract. The model estimates the residence times for the slowest compartment (rumen), the second slowest compartment (caecum/large intestine), and a transit time (tubular segments). Although ruminal and ileal cannulated animals were used it remained difficult to relate the parameter estimates unambiguously to the physical compartments. The transit time could not be solely ascribed to the tubular segments (intestines), but are to some extent also related to pre-abomasal related processes (reticulo-rumen, omasum). In addition, an attempt was made to relate animal and feed characteristics to the compartimental residence times, using multiple regression. The residence times of Cr were best related to feed and animal characteristics ($R^2 > 0.673$), followed by ^{13}CDM. Co gave poorest results ($R^2 < 0.379$).

In **chapter 7** (General Discussion) the relation between passage and degradation is discussed. From the preceeding chapters it appeared that the feed specific fractional passage rates were considerably lower than the fixed values currently adopted in the DVE-system, and adjustment of these values may have consequences. With the implications and the discussion on some future prospects for feed evaluation systems the thesis is concluded.

The following main conclusions are drawn from this thesis:

- Upon pulse dosing in the rumen, the excretion patterns of ^{13}C in the dry matter, the cell wall and the non-cell wall fractions are well fitted using the multi-compartmental model (MC-model) of Dhanoa *et al.* (1985). The observed asymptotic standard error of the parameter estimate, the coefficient of determination (R^2) and other curve fit evaluation methods like the Mean Prediction Error (MPE) showed that ^{13}C-markers did not differ from the traditionally used external markers Cr-NDF and Co-EDTA. This implies that ^{13}C can be regarded as a reliable marker. The MC-model generates good estimators for fractional passage rate constants describing the flow from the slowest compartment.

- The variation of estimated passage rates and retention times was rather high and some observations of individual animals had to be considered outliers, thus, making it difficult to establish significant effects. In future research it is recommended to use a larger number of experimental units.

- Fractional passage rates of the ^{13}C-marked fractions differed distinctly from that of the external markers Cr-NDF, which differed considerably from that of Co-EDTA. In all cases the liquid phase marker (Co) had significantly higher fractional passage rates than the particle phase markers (Cr, ^{13}CDM, ^{13}CNDR and ^{13}CNDS), which was in line with expectations. The internal markers had in general lower fractional passage rates than Cr, with the biggest contrasts between Cr and ^{13}CNDR. Therefore, the first hypothesis (page 5); "*the passage of the novel internal marker ^{13}C differs from the passage of traditionally used external markers*" has been accepted.

- Clear differences were observed between the passage behaviour of the dry matter, the cell wall fraction and the non-cell wall fraction. Assuming that the ^{13}C-enriched roughages are being subjected to the rumination and fermentation processes, it can be hypothesised that ^{13}C-markers give a better representation of the *in vivo* situation compared to the external markers. The compartmental and total residence times of the cell wall fraction (^{13}CNDR) upon ruminal pulse dosing were significantly longer than that of the dry matter (^{13}CDM) and the non-cell wall fraction (^{13}CNDS). In addition, the moment of peak concentration (PCT) and the transit time (TT) of ^{13}CNDR occured at a later moment, indicating a slower passage compared to ^{13}CDM and ^{13}CNDS. Therefore, the second hypothesis (page 5); "*different feeds and feed components show differences in passage behaviour*" has been accepted.

- The level of feed intake clearly affected the passage behaviour of the liquid and particle phase markers. When animals are subjected to a certain form of feed restriction the residence time will increase in all compartments, with largest effects measured in the rumen. Concomitantly, feed components are subjected to fermentation processes for a longer period, which eventually determines the site where nutrient become available, how they become available and when they become available. Based on the above, the part of the third hypothesis (page 5); "*influence of feed intake level on passage*" has been accepted.

- Grass silage of high digestibility (GSH) tended to higher fractional passage rates (thus shorter residence times) compared to grass silage of lower digestibility (GSL). The ^{13}C-marked fractions (DM, NDR, NDS) had sufficient power to distinguish between the passage rates of different silages and feed components. Therefore, the part of the third hypothesis (page 5); "*influence of diet quality on passage*" has been accepted.

- The fractional passage of ^{13}CDM and ^{13}CNDS are quite well related to the *in situ* determined fractional degradation rates. With regard to ^{13}CNDR no relation was observed. The number of observations is based on only four different experimental treatments and thus, these results should be regarded with carefull attention. However, compared to the relations as observed for the external markers, ^{13}C seems a promising tool to quantify the

relations between passage and degradation. Hence, the fourth hypothesis (page 5); "*the passage of feed particles from the rumen is largely related to the rate of degradation in the rumen*" may be accepted.

- The fixed values for passage as adopted in the DVE-system are not representative for the *in vivo* situation, and give in general an overestimation of the fractional passage rate. Besides, fixed values do not include sources of variation that are related to level of feed intake and diet quality as shown in the different animal experiments. The amount of by-pass feed protein (DVBE) will be overestimated and the amount of fermetable organic matter (FOM) will be underestimated and from that the amount of microbial protein (DVME). Therefore, with the current fixed values in use, the amount of DVE will be overestimated depending amongst others of the type and quality of the roughage.

SAMENVATTING

Binnen het huidige Nederlandse voederwaarderingssysteem wordt de energie- en eiwitbehoefte van melkvee beschreven door het VEM (Voedereenheid Melk) en DVE/OEB-systeem (Darm Verteerbaar Eiwit/ Onbestendige Eiwit Balans). Het DVE/OEB-systeem beschrijft het aanbod van eiwit dat de dunne darm bereikt waar het vervolgens verteerd kan worden. Deze aangeboden eiwitfractie kan worden opgesplitst in een darmverteerbare bestendige component (DVBE) en een darmverteerbare microbiële component (DVME). De mate waarin het voereiwit (DVBE) door de dunne darm opgenomen kan worden is gerelateerd aan de fermentatieve fractionele afbraaksnelheden van voercomponenten, fractionele passagesnelheden in de pens en de verteerbaarheden van voercomponenten in de dunne darm. De fermentatieve afbraak van digesta in de pens is voerspecifiek en kan worden vastgesteld middels *in sacco* experimenten (Tamminga *et al.*, 1994). Daarnaast wordt de beschikbaarheid van nutriënten bepaald door de snelheid waarmee de digesta door het maagdarmkanaal wordt getransporteerd. Over de hoogte en variatie van passagesnelheden is weinig bekend en binnen het DVE/OEB-systeem worden dan ook vaste waarden voor passagesnelheden vanuit de pens aangenomen, te weten 0,045/uur voor ruwvoeders en 0,060/uur voor krachtvoeders (Vérité *et al.*, 1987; CVB, 1991). Er komen echter steeds meer aanwijzingen dat ook passagesnelheden voerspecifiek zijn en onder meer afhankelijk zijn van ruwvoer type (Mambrini and Peyraud, 1994; Lund, 2002), ruwvoerkwaliteit (Bruining en Bosch, 1991; Rinne *et al.*, 1997), rantsoensamenstelling en niveau van voeropname (Tamminga *et al.*, 1989; Colucci *et al.*, 1990). Op basis hiervan is het aannemelijk dat het introduceren van voerspecifieke fractionale passagesnelheden mogelijk een verbetering geeft in het schatten van het moment waarop nutriënten beschikbaar komen met de daaruit voortvloeiende consequenties voor benutting.

Bij onderzoek naar passagesnelheden van voedercomponenten wordt veelal gebruik gemaakt van externe markeerstoffen zoals Cr-NDF (partikelfase) en Co-EDTA (vloeistoffase). In geval van Cr-NDF wordt de met Neutral Detergens uitgewassen en onverteerbare celwandfractie van stro met chroom geïmpregneerd (Udén *et al.,* 1980). Na een éénmalige toediening van deze markeerstoffen in de pens kunnen de fractionele passagesnelheden vastgesteld worden aan de hand van de afname van de markeerstofconcentratie in de pensinhoud, ileale chymus en/ of faeces. Hierbij wordt aangenomen dat de markeerstoffen representatief zijn voor het passagegedrag van de voerpartikels afkomstig uit het rantsoen. Het is echter aannemelijk dat de Cr-NDF fractie op basis van stro zich anders gedraagt dan de eigenlijke voerpartikels in het maagdarmkanaal en als zodanig geen goed beeld van de werkelijkheid geeft (Tamminga *et al.*, 1989). Voor het verkrijgen van voerspecifieke passagesnelheden is het wenselijk om (interne) merkers te gebruiken die de *in vivo* situatie weergeven.

Aan de hand van de bovengenoemde probleemstelling is in de zomer van 1997 een project gestart waarbij de passage van voerdeeltjes door verschillende compartimenten van het maagdarmkanaal bij melkkoeien onderzocht is. Voor het verkrijgen van informatie omtrent

voerspecifieke- en voercomponent-specifieke passagesnelheden is binnen dit project gebruik gemaakt van het stabiele isotoop van koolstof (^{13}C), dat als interne markeerstof fungeert.

In **hoofdstuk 2** wordt een beschrijving gegeven van een aantal veldproeven waarbij gras voorzien is van deze interne markeerstof. Gedurende deze zogenaamde verrijkingsprocedure werd de ^{13}C middels een natuurlijk verlopend assimilatieproces geïncorporeerd in met name de celwandfracties van het gras. Afhankelijk van de te beantwoorden vraagstelling in de verschillende dierproeven, werd het verrijkte gras als vers materiaal direct ingevroren (1x), dan wel ingekuild in nylon netten met de bulksilage en vervolgens ingevroren (3x). Van deze materialen zijn de residuele fracties bepaald na behandeling met een neutraal detergens (NDR), en een zuur detergens (ADR), en de lignine fractie (ADLR). In deze residuen kon vervolgens de hoogte van ^{13}C-verrijking worden vastgesteld. Overeenkomstig de literatuurgegevens bleek dat het verrijkingsniveau van de ADLR onder natuurlijke omstandigheden (achtergrondverrijking) een aantal eenheden $\delta^{13}C$ lager te liggen. Bij het artificieel verrijkte gras bleek dit effect versterkt naar voren te komen, waarbij de ADLR een significant lagere verrijking had ten opzichte van de overige fracties. Hieruit volgde de conclusie dat onder de gegeven experimentele condities, er een discriminatie bestaat tegen het inbouwen van ^{13}C in de ADL fractie. Dit effect is waarschijnlijk met name toe te schrijven aan de discriminatie tegen ^{13}C zoals die zich voordoen bij de formatie van de biosynthetische precursoren van lignine, phenylalanine en tyrosine.

Het primaire doel van de veldproeven was om een voor Nederlandse condities geschikte verrijkingsprocedure te ontwikkelen, waarmee voldoende verrijkt materiaal gewonnen kon worden voor de uitvoer van dierproeven met melkkoeien. In alle situaties bleek de procedure een adequate hoeveelheid verrijkt materiaal op te leveren.

In **hoofdstuk 3** wordt een tweetal dierproeven beschreven waarbij gekeken is naar het passagegedrag van de externe markeerstoffen Co-EDTA (Co) en Cr-NDF (Cr) en de passage van ^{13}C als interne markeerstof van de drogestof fractie (^{13}CDS), de celwandfractie ($^{13}CNDR$) en de niet celwandfractie ($^{13}CNDS$). De ^{13}C werd middels een natuurlijk verlopend assimilatieproces geïncorporeerd in met name de celwandfracties van gras. Vervolgens werd dit gras ingekuild. Deze met ^{13}C-verrijkte grassilage, werd vervolgens tesamen met Cr-NDF en Co-EDTA op verschillende plaatsen in het maagdarmkanaal van melkkoeien geïntroduceerd. De dieren, voorzien van een pens- en ileumcanule, kregen een grassilage/krachtvoer rantsoen met de verhouding 60:40 in de drogestof. Tijdens de eerste proef waren dieren nieuw melkt en hadden een totale dagelijks drogestof opname van 12,3 (sd = 0.3) kg/ dag (niveau Hoog). In de tweede proef werden dieren op 60% van het niveau van de eerste proef gevoerd (niveau Laag). De dieren ontvingen achtereenvolgens éénmalige doseringen van deze markeerstoffen in het ileum, de lebmaag en de pens. Vervolgens werden er faecale en ileale excretiepatronen vastgesteld van Cr, Co, ^{13}CDS, $^{13}CNDR$ en $^{13}CNDS$. Er werden geen significante verschillen in passagesnelheid tussen de merkers na ileum en lebmaag dosering waargenomen. Na een dosering in de pens bleek ^{13}CDS duidelijk langzamer te passeren dan de externe markeerstoffen. Een vergelijking tussen de met ^{13}C-verrijkte fracties liet tevens zien dat $^{13}CNDR$ langzamer

vanuit de pens passeert dan ^{13}CDS en ^{13}CNDS. De verschillen tussen deze fracties bleken echter niet significant te zijn. Een vergelijking tussen beide proeven liet daarnaast zien dat beperkt voeren resulteert in een verlaging van de passagesnelheid. De parameters welke gegenereerd zijn bij het modelmatig schatten van de excretiepatronen laten zien dat er duidelijke verschillen bestaan tussen het passagegedrag van de DS, NDR en NDS-fracties. Dit impliceert dat er verschillen bestaan tussen de mate en snelheid waarmee nutriënten beschikbaar komen in het maagdarmkanaal van koeien en dat ^{13}C als interne markeerstof de mogelijkheid biedt deze verschillen te onderscheiden. Zeker ten aanzien van het ontwikkelen van op nutriënten gebaseerde voederevaluatiesystemen lijkt ^{13}C als interne markeerstof een handvat te bieden bij het verkrijgen van voerspecifieke en voercomponentspecifieke passagesnelheden.

In **hoofstuk 4** is een vergelijking gemaakt tussen het passagegedrag van de externe markeerstoffen Co-EDTA en Cr-NDF met die van de intern gemarkeerde fracties ^{13}CDS, ^{13}CNDR en ^{13}CNDS in met gras gevoerde melkkoeien. Het rantsoen bestond uit vers gras aangevuld met krachtvoer in de verhouding 75:25 op basis van de drogestofopname. Het met ^{13}C-verrijkte verse gras werd, tesamen met de overige externe markeerstoffen, achtereenvolgens éénmalig gedoseerd het ileum, de lebmaag en de pens. Vervolgens werden de excretiepatronen van verschillende markeerstoffen (Co, Cr, ^{13}CDS, ^{13}CNDR en ^{13}CNDS) middels nonlineaire regressiemodellen gefit en vergeleken. Er bleken duidelijke verschillen te bestaan tussen het passagegedrag van de externe en interne merkers en tussen de DS, NDR en NDS-fracties onderling. Er waren geen significante verschillen in passagesnelheid tussen de merkers na ileum en abomasum dosering. Wel neigde na abomasum dosering Co tot een snellere passage dan Cr en ^{13}CDS. Bij dosering in de pens was de passagesnelheid van Co significant hoger dan die van de andere markeerstoffen. Cr neigde naar een hogere passagesnelheid dan ^{13}CDS en ^{13}CNDR. Ook binnen de met ^{13}C gelabelde fracties waren er verschillen in uitscheidingscurves. Passagesnelheden van ^{13}CNDR na een dosering in de pens neigden naar lagere waarden dan die van ^{13}CDS en ^{13}CNDS. Analoog hieraan neigde de celwandfractie tot langere retentietijden in het maagdarmkanaal. Ook deze resultaten impliceren dat er verschillen bestaan tussen de mate en snelheid waarmee nutriënten beschikbaar komen in het maagdarmkanaal van koeien en bovendien dat de plaats in het maagdarmkanaal waar nutriënten beschikbaar komen verschilt per fractie. Tevens geven deze resultaten aan dat ^{13}C als interne markeerstof het onderscheidend vermogen heeft om de verschillen in passagegedrag tussen de verschillende fracties te kwantificeren, dit in tegenstelling tot de externe markeerstoffen die deze mogelijkheid niet hebben.

In de dierproef beschreven in **hoofdstuk 5** zijn drie HF melkkoeien gebruikt die voorzien waren van een pensfistel en een ileumcanule. Bij aanvang van de proef waren dieren in vroege lactatie en werden volgens lot toegekend aan een der rantsoenbehandelingen; een grassilage met lage verteerbaarheid (GSL) versus en grassilage met hoge verteerbaarheid (GSH). Het ruwvoer werd aangevuld met een speciaal samengesteld krachtvoeder in een vaste verhouding (54:46). Ook hier ontvingen dieren éénmalige pulsdoseringen in het ileum, de lebmaag en de pens met Cr-NDF en Co-EDTA en met één der ^{13}C-verrijkte grassilages, waarna de faecale en

ileale excretiepatronen van Co, Cr, ^{13}CDS, ^{13}CNDR en ^{13}CNDS werden vastgesteld. Faecaal bepaalde fractionele passagesnelheden volgend op een ileum of lebmaag dosering lieten geen verschillen zien tussen markeerstoffen onderling en het verschil in verteerbaarheid van de silages had geen meetbare invloed op de passage van markeerstoffen in het post-ruminale deel van het maagdarmkanaal. Een dosering in de pens bleek duidelijk lagere fractionele passage constanten voor ^{13}CDS en ^{13}CNDR te geven ten opzichte van Cr en Co. De gecombineerde informatie over het moment waarop de hoogste markeerstofconcentratie wordt gevonden, de aanlooptijd, de verblijftijden in de verschillende compartimenten en in het totale maagdarmkanaal, lieten grotere silageeffecten zien voor de interne (^{13}C) markeerstoffen dan voor de externe markeerstoffen (Cr en Co). Uit de gegevens werd dan ook geconcludeerd dat ^{13}C een hoger onderscheidend vermogen heeft om verschillen in verteerbaarheid tussen rantsoenen aan te tonen dan Cr en Co. In relatie tot afbraak en 'total clearance' van de vaste fase bleken de ^{13}C-markeerstoffen rationelere waarden te leveren dan Cr.

Ten aanzien van het modelmatig beschrijven van excretiepatronen kan een onderscheid gemaakt worden tussen de stochastische en deterministische benadering. In **hoofdstuk 6** worden een aantal van deze stochastische modellen, variërend in zogenaamde gamma-tijdafhankelijke factoren (gamma-N, 1 tot 5), vergeleken met een, in onze studies gebruikte, deterministische multicompartimenten model. Een belangrijk punt van afweging is dat de keuze van het model van grote invloed is op de interpretatie van het excretiepatroon. Wordt in stochastische modellen de invloed van het dikke darm compartiment als ondergeschikt beschouwd, en alszodanig niet afleidbaar uit het excretiepatroon, in het deterministische model wordt het caecum/ dikke darmcompartiment als een belangrijk vertragend compartiment beschouwd.

Het samenvoegen van alle excretiepatronen van de verschillende markeerstoffen uit de afzonderlijke proeven gaf een krachtige dataset met de mogelijkheid om de verschillen en de robuustheid van modellen te vergelijken. Met betrekking tot het fitten van de excretiepatronen bleek het deterministische model goede resultaten te geven. Stochastische modellen van lagere gamma-orden (gamma-N < 3) gaven vaker slechtere fits dan hogere gamma-N modellen. Verder bleek dat de excretiepatronen van de interne markeerstoffen na ileum en lebmaag doseringen relatief minder slechte fits te geven dan die van Co en Cr. Het percentage slechte fits voor faecale en ileale excretiepatronen liet geen verschil tussen markeerstoffen zien. De conclusie was dat de interne markeerstoffen mathematisch goed zijn te fitten en in vergelijking met de externe markeerstoffen goede resultaten geven. Het deterministische model gaf goede fits en bleek zeer robuust ten opzichte van de overige modellen.

Aan de hand van het deterministische model is vervolgens getracht om compartimentele verblijftijden zoals geschat uit de faecale excretiepatronen, te relateren aan fysieke compartimenten. Het model schat verblijftijden voor het traagste compartiment (pens, CMRT1), het op een na traagste compartiment (caecum/ dikke darm; CMRT2) en een transit tijd (tubulaire segment; TT). Ondanks het gebruik van ileum- en pensgecanuleerde dieren bleek het moeilijk om excretiepatronen eenduidig te relateren aan compartimenten. De transit

tijd geschat door dit model kan niet enkel worden toegeschreven aan de tubulaire segmenten (dunne darm), maar zijn tevens gerelateerd aan processen die zich pre-abomasaal (pens, netmaag, boekmaag) afspelen. Tevens is een aanzet gegeven om middels multiple regressie dier en voerkarakteristieken te relateren aan de compartimentele verblijftijden. Hierbij bleken de verblijftijden van Cr gemiddeld het beste gerelateerd te zijn aan voer en dierkarakteristieken ($R^2 > 0.673$), gevolgd door ^{13}CDS. Co gaf slechte resultaten ($R^2 < 0.379$).

In **hoofdstuk 7** (General Discussion) wordt ingegaan op de hypothese dat de passage vanuit de pens gerelateerd is aan de fermentatieve afbraak. Verder bleek uit de voorgaande hoofdstukken dat de voerspecifieke fractionele passagesnelheden beduidend lager liggen dan de huidig gehandhaafde passageconstanten in het eiwitwaarderingssysteem. Het bijstellen van deze waarden zal de nodige implicaties met zich meebrengen. Met de gevolgen hiervan en toekomstperspectieven met betrekking tot voederwaarderingssystemen wordt het proefschrift afgesloten.

De belangrijkste conclusies die op basis van het onderzoek getrokken zijn:

- Na toediening in de pens zijn excretiepatronen van ^{13}C, als markeerstof van de drogestof, de celwand en de niet-celwand fracties, goed te beschrijven met het multi-compartimenten model (MC-model) van Dhanoa *et al.* (1985). De gevonden asymptotische standaard fouten van gemiddelde parameterschattingen, de determinatie coëfficiënten (R^2) en overige curvefit-evaluatiemethoden zoals de Mean Prediction Error (MPE) laten geen afwijkingen zien ten opzichte van de meer traditionele markeerstoffen zoals Cr-NDF en Co-EDTA. Dit duidt erop, dat ^{13}C een betrouwbare interne markeerstof is. Het MC-model genereert goede schatters voor de passagesnelheden van fracties door het meest trage compartiment van het maagdarmkanaal van koeien, ongeacht het soort ruwvoer, het niveau van voeropname of de verteerbaarheid van het verstrekte (ruw)voer.

- De variatie in geschatte passagesnelheden en retentietijden is tamelijk hoog, en soms konden waarnemingen aan individuele dieren niet meegenomen worden. Daardoor konden er niet altijd significante effecten ($P < 0.05$) worden aangetoond. Het verdient aanbeveling in de toekomst meer herhalingen per dier uit te voeren of een groter aantal dieren in te zetten.

- Passagesnelheden van de hier gebruikte op ^{13}C gebaseerde interne markeerstoffen verschilden qua gedrag duidelijk van de externe markeerstof Cr-NDF, dat op zijn beurt weer duidelijk verschilde van Co-EDTA. In alle gevallen had de markeerstof van de vloeistof fase (Co) significant hogere passagesnelheden dan de deeltjes fase markeerstoffen (Cr en ^{13}C in de DS, NDR en NDS), wat ook in lijn is met de verwachting. In nagenoeg alle gevallen hadden de interne markeerstoffen een duidelijk lagere

passagesnelheid ten opzichte van Cr, waarbij de grootste verschillen werden gevonden tussen Cr en ^{13}CNDR. Hiermee wordt de hypothese onder punt 1 (pag. 5), "*The passage of the novel internal marker ^{13}C differs from the passage of traditionally used external markers*", aangenomen.

- Er zijn duidelijke verschillen aangetoond tussen het passagegedrag van de drogestof, de celwandfractie en de niet-celwandfractie. Ervan uitgaande dat de ^{13}C-verrijkte ruwvoeders onderhevig zijn aan herkauw- en fermentatieprocessen mag gesteld worden dat ^{13}C de *in vivo* situatie beter weergeeft dan de externe merkers. De compartimentale en totale verblijftijden van de celwandfractie (^{13}CNDR) na een dosering in de pens waren significant langer dan die van de drogestof (^{13}CDS) en de niet-celwandfractie (^{13}CNDS). Analoog hieraan vielen de PCT (moment van piekconcentratie) en de TT (aanlooptijd) voor ^{13}CNDR op een later moment wat duidt op een tragere passage ten opzichte van ^{13}C in de DS en NDS. Hiermee wordt de tweede hypothese (pag. 5), "*Different feeds and feed components show differences in passage behaviour*", aangenomen.

- Het niveau van voeropname had een duidelijke invloed op het passagegedrag van zowel vloeistof als vaste fase markeerstoffen. Indien dieren een voerrestrictie opgelegd krijgen zal in alle compartimenten van het maagdarmkanaal, maar met name in de pens, de retentietijd toenemen. Dit heeft tot gevolg dat voedercomponenten in de pens langer bloot staan aan het fermentatieproces wat weer van invloed is op de plaats waar, en de tijdstippen waarop de componenten beschikbaar komen voor het dier. Hiermee wordt een onderdeel van de derde hypothese (pag. 5), te weten; "*influence of feed intake level on passage*", aangenomen.

- Hoogverteerbare grassilage (GSH) neigde naar hogere passagesnelheden en hieraan gerelateerd kortere verblijftijden dan laagverteerbare grassilage (GSL). De met ^{13}C gemarkeerde fracties (DS, NDR, NDS) hadden een voldoende onderscheidend vermogen om verschillen tussen silages en voercomponenten onderling aan te tonen. Hiermee wordt het onderdeel van de derde hypothese (pag. 5), te weten; "*influence of diet quality on passage*", aangenomen.

- De fractionele passage van ^{13}CDS en ^{13}CNDS laten redelijke tot goede verbanden zien met de *in situ* vastgestelde afbraakconstanten. De mate waarin de passage van ^{13}CNDR gerelateerd is aan de afbraak, is echter laag. Omdat het aantal waarnemingen gebaseerd is op een beperkt aantal dierproeven moet hier met gepaste voorzichtigheid naar gekeken worden. Op grond van de bevindingen met externe markeerstoffen lijkt ^{13}C een veelbelovende markeerstof om relaties tussen passage en afbraak te kunnen kwantificeren, waarmee de vierde hypothese (pag. 5), "*The passage of feed particles from the rumen is largely related to the rate of degradation in the rumen*", aangenomen wordt.

- De vaste waarden voor passage, zoals aangenomen in het DVE-systeem geven een niet geheel juist beeld van de *in vivo* situatie en leiden meestal tot een overschatting. Vaste passagewaarden nemen niet de variatie mee die ontstaat door bijv. verschillen in voerniveau en voerkwaliteit, zoals in dit project aangetoond. Hieruit volgt dat het aandeel bestendig voereiwit wordt overschat en daarmee ook het aandeel DVBE. De hoeveelheid FOS wordt onderschat alsmede de daaruit berekende DVME. Als resultante zal, afhankelijk van het type en kwaliteit ruwvoer, de hoeveelheid DVE in meer of mindere mate overschat worden bij huidig aangenomen vaste passagesnelheden.

LIST OF PUBLICATIONS

Scientific papers

Pellikaan, W.F., H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven, and S. Tamminga. 2004. ^{13}C-enrichment of cell wall fractions of rye grass (*Lolium perenne* L.) and implications for its use as digesta marker. *Submitted to Journal of Animal Science*.

Pellikaan, W.F., J. Dijkstra, S. Tamminga, H. Boer, S.C.W. Lammers-Wienhoven, W.J.H. van Gestel and G. Hof. 2004. Passage of ^{13}C-labelled grass silage through the gastro-intestinal tract of dairy cows at two levels of feed intake. *Submitted to Journal of Animal Science*.

Pellikaan, W.F., J. Dijkstra, G. Hof, B.M. Tas, H. Boer, and S. Tamminga. 2004. Passage of ^{13}C-labelled grass through different compartments of the gastro-intestinal tract of dairy cows. *Submitted to British Journal of Nutrition*.

Pellikaan, W.F., S. Tamminga, G. Hof, H. Boer, M. Roordink, and J. Dijkstra. 2004. Passage of ^{13}C-labelled grass silages differing in quality through the gastro-intestinal tract of dairy cows. *Submitted to Journal of Dairy Science*.

Abstracts

Pellikaan, W.F., H. Boer, J. Dijkstra, J., G. Hof, V.V.A.M. Schreurs, S. Tamminga, and J. Vos. 1999. Passage dynamics of [^{13}C]-enriched feed particles in grass silage fed dairy cows. South African Journal of Animal Science, 29: 274-275.

Pellikaan, W.F., H. Boer, J. Dijkstra, J., G. Hof, V.V.A.M. Schreurs, S. Tamminga, and J. Vos. 1999. Passage van ^{13}C-verrijkte voerdeeltjes uit grassilage door het maagdarmkanaal van melkkoeien. Proceedings 24ste Studiedag Nederlandstalige Voedingsonderzoekers, Gent, 9 april: 38-39.

Pellikaan, W.F., H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven, and S. Tamminga. 2000. ^{13}C-enrichment of rye grass and its use as digesta marker in ruminant nutrition research. Grassland Science in Europe, 5: 325–327.

Pellikaan, W.F., H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven, and S. Tamminga. 2000. Passage van ^{13}C-verrijkte grassilage, Co-EDTA en Cr-NDF door diverse compartimenten van het maagdarmkanaal bij melkkoeien bij een hoog en laag niveau van voeropname. Proceedings 25ste Studiedag Nederlandstalige Voedingsonderzoekers, Wageningen, 28 april: 38-39.

Pellikaan, W.F., H. Boer, J. Dijkstra, G. Hof, S.C.W. Lammers-Wienhoven, and S. Tamminga. 2000. Passage dynamics of ^{13}C-enriched grass silage in dairy cows fed at high and low level of feed intake. Book of abstracts, EAAP - 51st annual meeting, The Hague, 21-24 August 2000, 6: 131.

Reports (eindrapportages)

Pellikaan, W.F., J. Dijkstra, H. Boer, G. Hof, S.C.W. Lammers-Wienhoven, S. Tamminga, en J. Vos. 2000. Passage van ^{13}C-verrijkte voerdeeltjes uit grassilage door diverse compartimenten van het maagdarmkanaal bij melkkoeien bij een hoog en laag niveau van voeropname. WVH-319: p 17.

Pellikaan, W.F., J. Dijkstra, H. Boer, G. Hof, S.C.W. Lammers-Wienhoven, en S. Tamminga. 2002. Passage van de droge stof, celwand en niet-celwand fractie van met ^{13}C-verrijkte grassilage door diverse compartimenten van het maagdarmkanaal van melkkoeien bij twee niveaus van voeropname. WVH-02-16: p 15.

Pellikaan, W.F., J. Dijkstra, H. Boer, G. Hof, S.C.W. Lammers-Wienhoven, en S. Tamminga. 2002. Passage van Co-EDTA, Cr-NDF en met ^{13}C-verrijkt gras door diverse compartimenten van het maagdarmkanaal bij grasgevoerde koeien. WVH-02-17: p 19.

Pellikaan, W.F., J. Dijkstra, G. Hof, H. Boer, M. Roordink, S. Tamminga. 2003. Passage van Co-EDTA, Cr-NDF en ^{13}C-verrijkte fracties van twee grassilages van verschillende verteerbaarheid door diverse compartimenten van het maagdarmkanaal van melkkoeien. WVH-03-91: p 24.

Thesis reports (afstudeerscripties en stageverslagen)

Hemmer, S. (1999): De pH en het gehalte fermentatieproducten in de pensvloeistof van melkvee, bij het voeren van een grassilage met een hoog en laag ruwe celstof gehalte. Afstudeerscriptie t.b.v. de Hogeschool Delft.

Jorna, F. (2000). Vergelijking van de geschatte passagesnelheden van NDR en DS door middel van ^{13}C analyse. Doctoraal scriptie Diervoeding, Wageningen universiteit.

Khot, D. (1999): Relationship between digestion, digestibility, volatile fatty acid ratio and milk fat content. MSc. scriptie Diervoeding, Wageningen universiteit.

Lange, A. de (1998). Passage schatting met interne en externe markeerstoffen in melkkoeien. Doctoraal scriptie Diervoeding, Wageningen universiteit.

Lopes, J.C. (1999): Effects of feed quality and composition on C/N ratio in the faeces and at the level of the large intestine in dairy cows. Afstudeerscriptie t.b.v. de Universidade de Tras-os-Montes e Alto Douro, Vila Real, Portugal.

Mast, H. van der (2000). Passage van voerdeeltjes door diverse compartimenten van het maagdarmkanaal bij melkkoeien. Passageschatting met interne en externe markeerstoffen in melkkoeien met een beperkte voeding. Doctoraal scriptie Diervoeding, Wageningen universiteit.

Roordink, M. (1999): Schatten van passagesnelheden: Toepasbaarheid van niet lineaire compartiment modellen om bij melkkoeien faecale en ileale patronen van markeerstofconcentraties te beschrijven. Doctoraal scriptie Diervoeding, Wageningen universiteit.

Tas, B. (2000). Afbraak en passage van vers gras in de pens bij melkkoeien. Passage geschat met Co-EDTA, Cr-NDF en [^{13}C]-gelabeld gras. Doctoraal scriptie Diervoeding, Wageningen universiteit.

Teunissen, A. (1998). Bepalen van de afbreekbaarheid en degradatiesnelheid van voeders in de pens van melkkoeien middels *in-sacco* methode. Scriptie t.b.v. de Agrarische Hogeschool Larenstein Deventer.

Uiterwaal, S. (1999). *In sacco* afbraakexperiment van 2 grassilages, krachtvoer en sojaschroot. Scriptie t.b.v. Agrarische Hogeschool Den Bosch.

BIOGRAPHY

Wilbert Frans Pellikaan was born on September 15th 1967 in Heerenveen as a son in a local docters familie in Nieuwehorne, a village in the nothern part of the Netherlands. Growing up on their neighbour's farm and his fathers' affinity with farming life from bygone days, his interest in agriculture was triggerd from childhood on. After the local primary school he went to secondary school in Heerenveen. During this time at the age of 12 he was introduced into the world of harnass racing (trotters) and spent most of his spare time at the 'entrainement' of T. Smeding. For the coming 10 years the work with trotters was his passion. The, for Dutch conditions novell approach regarding the training and handling of animals as introduced by this man, as well as working with the people in his team have been of considerable influence.

In 1983 he went to the Modern Agricultural School (Drachten) with respective traineeships on a dairy farm (Nieuwehorne), an entrainement (Grabensee, Austria) and an arable farm (Hijum). From 1986 to 1991 he studied Dutch Agriculture (BSc) at the Agricultural College in Friesland, during which he did a traineeship on a dairy farm (Nullawarre, Australia) and an internship within a para-statal company in Kenya (Agricultural Development Corporation, stationed in Kitale). Under draft 91-5 he spend 1 year of compulsory military service in Seedorf (Germany) and started in September 1992 the program in animal sciences at the Wageningen Agricultural University. He completed his study in 1996 with a minor subject in Immunology (atrophic rhinitis in piglets) and two major subjects in tropical animal husbandry & physiology (compensatory growth in ram lams), and in agronomy (woody weeds infestation in Australian open woodlands). The latter subject was conducted within the research organisation CSIRO (Townsville, Australia).

After temporary work at the group of Human and Animal Physiology in February 1997 he got a temporary position at the group of Animal Nutrition. In June 1997 the Ph.D-project started as described in the current thesis. Subsequently, in November 2001 a post-doctoral study started that, at the current moment (September 2004) is near completion.

During his Ph.D.-period he met Joana, to whom he married in November 2001 and by now are raising two excellent daughters.

LEVENSLOOP

Wilbert Frans Pellikaan is geboren op 15 september 1967 te Heerenveen als zoon uit een huisartsenfamilie woonachtig te Nieuwehorne. Het opgroeien op de boerderij van de buren alsmede de affiniteit van zijn vader met het boereleven van vroeger, maakten dat hij het wel en wee binnen de agrarische sector van jongsaf meekreeg. Op zijn twaalfde werd hij via het entrainement van dhr. T. Smeding geïntroduceerd in de Nederlandse Draf- en Rensport, en gedurende de hierop volgende 10 jaar vormde het werken met harddravers zijn passie. De vooruitstrevende kijk op trainen en (be)handelen van paarden van deze persoon, alsmede het werken met de mensen binnen zijn team zijn van grote invloed geweest. Na het doorlopen van de openbare MAVO school te Heereneveen ging hij in september 1983 naar de Rijks Middelbare Landbouwschool te Drachten, waar achtereenvolgens stages volbracht werden op een melkveehouderijbedrijf (Nieuwehorne), een paardenhouderijbedrijf (Grabensee, Oostenrijk) en een akkerbouwbedrijf (Hijum). Vanaf 1986 tot 1991 werd de studie Nederlandse landbouw aan de Agrarische Hogeschool van Friesland doorlopen, met achtereenvolgend een stage op een melkveehouderijbedrijf (Nullawarre, Australië) en een onderzoeksstage bij de Agricultural Development Corporation (Kitale, Kenia). Na het vervullen van 1 jaar militaire dienstplicht in Seedorf (Duitsland) begon hij in september 1992 aan het toenmalige doorstroomprogramma binnen de richting zoötechniek van de Landbouwuniversiteit. De studie werd afgerond in juni 1996, met afstudeervakken in de immunologie (atrofische rhinitis bij varkens), tropische veehouderij & fysiologie (compensatoire groei bij ramlammeren) en agronomie (woody weeds in Austalian open woodlands). Met betrekking tot het laatste onderwerp verbleef hij voor 6 maanden bij de CSIRO in Townsville, Australië. Na een tijdelijk dienstverband bij de leerstoelgroep Fysiologie van Mens en Dier begon hij per februari 1997 als tijdelijk medewerker bij de leerstoelgroep Diervoeding. Hier startte in juni 1997 het AIO-project waarvan melding gemaakt wordt in dit proefschrift. Aansluitend aan het onderzoek is per november 2001 een post-doc project gestart wat zich op dit moment (september 2004) in een afrondingsfase bevindt. Tijdens zijn AIO-periode leerde hij zijn vrouw Joana kennen, met wie hij inmiddels twee fantastische dochters heeft.

The study described in this dissertation was co-financed by the Dutch Commodity Board of Feedstuffs (PDV).

Printed by Universal Press in Veenendaal, with sponsoring of:
Wageningen University, Wageningen, The Netherlands.

Training and Supervision Plan		**Graduate School WIAS**
Name PhD student	Wilbert Pellikaan	
Project title	*Passage of feed particles through different compartments of the gastro-intestinal tract of dairy cows*	
Group	Animal Nutrition	
Daily supervisor(s)	Dr.ir. G. Hof; Dr.ir. J. Dijkstra	
Supervisor(s)	Prof.dr.ir. S. Tamminga	
Project term	from June 1996	until July 2001
Submitted	04 February 2004	first plan / midterm / **certificate**

One credit point (cp) equals a study load of approximately 40 hours.

PREVIOUS EDUCATION	
MSc degree obtained at	Wageningen University, Wageningen, June 1996
Areas of expertise	**Immunology, Ruminant Physiology, Agronomy (tropical range lands)**

EDUCATION AND TRAINING (minimum 21 cp, maximum 42 cp)		
The Basic Package (minimum 2 cp)	year	cp
WIAS Introduction Course (mandatory, 1 cp) or WIAS Common Course (2 cp)	1997	2.0
Course on philosophy of science and/or ethics (mandatory, 1 cp)	1998	1.0
Subtotal Basic Package		**3.0**
Scientific Exposure (conferences, seminars and presentations, minimum 5 cp)	year	cp
Seminars & Workshops (WIAS or other)		
- 23ste studiedag Ned.talige Voed. Ond.	1998	0.2
- Feed technology.	1998	0.1
- IRMS Symposium (oral presentation no. 1)	1998	0.1
- WIAS science day '98 (poster no.2)	1998	0.2
- 24ste studiedag Ned.talige Voed. Ond. (oral presentation no. 3)	1999	0.2
- Nutrition and gut health. (WIAS seminar +)	1999	0.4
- WIAS science day '99.	1999	0.2
- Vth Int. workshop on modeling nutrient utilization. (poster no. 5)	1999	0.6
- 25ste studiedag Ned.talige Voed. Ond. (oral presentation no. 4)	2000	0.2
- Symposium on cummulative gasproduction. (EAAP2000)	2000	0.4
- WIAS science day '00. (poster no. 5 and 6)	2000	0.2
- WIAS science day '01. (oral presentation no. 8)	2001	0.2
- Organic Farming: a challenge for APS-research. (WIAS seminar +)	2000	0.3
International conferences *(minimum 2 cp)*		
- 5th Zodiac symposium. Regulation of feed intake.	1998	0.6
- IX ISRP symposium (South Africa). (poster no. 5)	1999	1.2
- 6th biennial symposium on ruminant nutrition (South Africa).	1999	0.2
- EAAP2000. (poster presentation no. 7)	2000	0.8
Presentations (minimum 4 original presentations of which at least 1 oral, 0.5 cp each)		
- IRMS Symposium (oral presentation no. 1)	1998	0.5
- WIAS science day '98 (poster no.2)	1998	0.5
- 24ste studiedag Ned.talige Voed. Ond. (oral presentation no. 3)	1999	0.5
- 25ste studiedag Ned.talige Voed. Ond. (oral presentation no. 4)	2000	0.5
- IX ISRP symposium (South Africa) (poster no. 5)	1999	0.5
- EGF2000 (poster no. 6)	2000	0.5
- EAAP2000 (poster presentation no. 7)	2000	0.5
- WIAS Science day '01 (oral presentation no. 8)	2001	0.5
Subtotal International Exposure		**10.1**

Name PhD student	Wilbert Pellikaan			
In-Depth Studies			year	cp
Disciplinary and interdisciplinary courses				
- Two phase liquid and solid flow (PAON)			1997	0.2
- Simulation models in feed evaluation (EAAP)			2000	0.6
- Stable isotope studies of nutrient dynamics (WIAS).		**A**	2000	1.0
Advanced statistics courses (optional)				
- Statistical Course: Robust Statistics (WIAS)			1998	1.0
- Study Group Time Serie Analyses (Mathematics Group)		**B**	2000	0.6
- Statistical Course: Experimental Design (WIAS)		**C**	2000	0.4
Subtotal In-Depth Studies				**3.8**
Professional Skills Support Courses (minimum 2 cp)			year	cp
WIAS Course Techniques for Scientific Writing (advised)			1998	0.5
Use of Laboratory Animals (mandatory when working with animals, 3 cp)			1997	3.0
Subtotal Professional Skills Support Courses				**3.5**
Research Skills Training (apart from carrying out the PhD project, optional)			year	cp
-Writing proposal post-doc (including budgetting, oral presentation financers, etc.)			2000	3.0
Subtotal Research Skills Training				**3.0**
Didactic Skills Training (optional)			year	cp
- Preparing case study topic stable isotope studies of nutrient dynamics (WIAS).			2000	0.5
Supervising practicals and excursions				
- Biologie dierlijke productie			98-'00	1.5
Supervising MSc theses (maximum 1 cp per MSc student)				
A. de Lange	Doctoraal student		1998	1.0
D. Khot	MSc student		1999	1.0
M. Roordink	Doctoraal student		1999	1.0
H. van der Mast	Doctoraal student		2000	1.0
B. Tas	Doctoraal student		2000	1.0
F. Jorna	Doctoraal student		2000	1.0
K. Verburg	Doctoraal student		2003	1.0
A. Teunissen	Halfjaar stage AHS Dronten		1998	0.5
J.C. Cerqueira-Lopes	End thesis Universidade de tras os montes e alto douro		1999	0.5
S. Hemmer	Eindscriptie AHS Delft		1999	0.5
S. Uiterwaal	Halfjaar stage AHS den Bosch		1999	0.5
Tutorship				
- Landbouworientatie			1998	0.5
- Boerderijproject			1999	0.5
Subtotal Didactic Skills Training				**12.0**
Management Skills Training (optional)			year	cp
Membership of boards and committees				
Onderwijscie. WIAS			97-'99	3.0
Ad hoc cie. In-sacco			99-'01	2.0
Subtotal Management Skills Training				5.0
Education and Training Total (minimum 21 cp, maximum 42 cp)				**40.4**

Title of presentations

1. The use of [13C]-enriched feedstuffs to estimate passage rates in the digestive tract of dairy cows
2. Passage dynamics of Cr-NDF, Co-EDTA and 13C-labeled grass silage through different compartments of the gastro intestinal tract in early lactation dairy cows
3. Passage van [13C]-verrijkte voerdeeltjes uit grassilage door het maagdarmkanaal van melkkoeien
4. Passage van [13C]-verrijkte voerdeeltjes uit grassilage door diverse compartimenten van het maagdarmkanaal bij melkkoeien bij een hoog en laag niveau van voeropname
5. Passage dynamics of [13C]-enriched feed particles in grass silage fed dairy cows
6. 13C-enrichment of rye grass and its use as digesta marker in ruminant nutrition research
7. Passage dynamics of 13C-enriched grass silage in dairy cows fed at high and low level of feed intake