GRAZING TIME: THE MISSING LINK

A study of the plant-animal interface by integration of experimental and modelling approaches

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Pablo Chilibroste

Proefschrift

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PREFACE

Decided to write a book (this Thesis), I have noticed that a PREFACE is necessary to make it complete. Today, I have been the whole morning in front of the keyword to do the job, but nothing appeared yet in the screen. It happens, that it is arduous to write down uninterrupted, when you have such a large number of things, to remember, to re-create, to taste, to miss and even to let drop a tear. Besides, almost nothing to regret. Anyway, in this Preface I would like to mention people that have been important for the completion of this study.

First of all, I would like to say thanks to my supervisor and **captain** of the VV (Animal Nutrition) team, Professor S. Tamminga. Dear Seerp, I could acknowledge your support, your wise guidance, your clever suggestions, your time, your refined sense of humor and even your enormous patient wit me. However, I would like to stress an aspect that I do esteem the most: your friendship. Not doubt I will do my best to remain as a member of the *"Seerp warriors" tribe.*

Also, I would like to acknowledge two members of the **defensive** line of the VV team: Germ Hof and Huug Boer. Germ gave me the opportunity to appreciate the "real" Dutch land and to enjoy multiple fruitful meetings. Huug, did the necessary polishing, to let me go and move within the Lab. Subsequently, he induced me the good habit to carefully check every simple procedure and I did so, even with the *more elementary one's...*

A paragraph to the strong and brave **mead-field** of the VV team. Just to mention the hard core: Martin, Rene and Thomas, Josien and Maria, Jane-Martine and colleagues in the Lab, Tamme and Peter, Malu, Marianne and Barbara. The multiple and opportune interventions of Josien, Tamme and Peter, solve us many troubles, within and outside of the University. To all of you, many, many thanks.

Special acknowledgment, to my co-promotor (Jan Dijkstra) and paraninfs (Walter Gerrits and Peter van der Togt). Our relationship with Jan has experienced a positive exponential slope, and I hope Jan, that this function will not exhibit a visible asymptote soon. With Walter, we have shared daily science, football (theory and practice), talks about life philosophy, expectations and uncertainties, and all these things over which you can build a close friendship. Peter has suffered me from the real first day (in his office but also in his kitchen at home..) and has been an invaluable support.

With Menno, Harmen, Carina, Jos and Jacob (PhD students) we have been in the same "carrousel" for a while and we learn from each other in many occasions. The fruitful talks with Menno between 4 and 7 o'clock a.m. (thanks Barbara), are still fresh in my memory. I have also a very good memory of "my MSc students", Roelof, Bert, Tinh, Maartje, Gera and André,: thanks you all for your pleasant, enthusiastic and responsible way of working. The collaborative and fruitfully work done with Sip and Christel, constituted a landmark during my stay in WAU, and it deserve further steps. My acknowledge to direction and personnel of the experimental farm "De Ossekampen" where the grazing trial were carried out.

Diego, Margarita, Fernando, Gabriel y Manuel han estado siempre presentes. El saberse con amigos (no importa a cuantos km se encuentren), da mucha fortaleza y coraje para ir hasta el fin: a ustedes, mil gracias por estar siempre ahí.

Quiero agradecer también a los colegas de producción animal EEMAC, con quienes hemos compartido cursos, hipótesis, experiencias y ahora nuevos proyectos. Un reconocimiento especial a Fernando Rodríguez, quien nos *zambulló* dentro del rumen y más importante aún, nos dio pistas por donde *bucear*.

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Por último debo confesar que esta Thesis tiene tres coautores ocultos: **Noel, Elena y Pedro**, compañeros incondicionales del proyecto más largo, el de la vida. Noela, tu amor, entereza y entrega sin miramientos, han sido la única y fundamental certeza en todo este recorrido. Ele, sorry por haberte andando saltando de escuela en escuela y de amigos en amigos, por tanto tiempo. Pedri, sin dudas, tu has constituido el hallazgo más relevante de nuestra estadía en Wageningen. Los amo.

Pablo Chili

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Cover: Fernando Cetrulo & Helena and Alvaro Heinzen

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Chilibroste, P. 1999. Grazing Time: the missing link: a study of the plant-animal interface by integration of experimental and modelling approaches. A series of grazing (chapters 2, 3, 5 and 6) *in-vitro* (chapter 4) and modelling trials (chapters 1 and 7) were combined with the following objectives: a) to gain insight in the main mechanisms controlling dry matter intake (**DMI**), intake rate (**IR**) and grazing time (**GT**), during the first grazing session after a.m. milking, b) to judge the relative importance of rumen fill and the concentration of fermentation products in the rumen liquor as candidates to signal the end of the grazing sessions and c) to develop new and modify and evaluate existing simulation model, to operate under non-steady state conditions with the aim to predict DMI, rumen fermentation and supply of nutrients. Increasing the length of the allowed grazing time significantly increased DMI ($P<0.01$), the proportion of time spent actively eating ($P<0.01$) and DM rumen pool size after grazing ($P<0.05$). The allowed grazing time did not have any significant effect on total and liquid rumen pool sizes after grazing but did have ($P<0.05$) on DM and OM (slope, 0.5 kg h^{-1}) rumen pool sizes. DMI as well as GT were greater after a starvation period of 16.5 h and were reduced by the presence in the rumen of indigestible material ($P<0.01$). The interaction between starvation time and rumen fill before grazing on GT, although not significant ($P<0.06$), supports the idea of a combination of signals controlling meal size under grazing conditions. Grazing time did not follow a significant trend with period of regrowth. In this trial, rumen fill (as represented by total, DM or NDF rumen pools size), volatile fatty acids (**VFA**), ammonia, pH and osmotic pressure as individual variables were not correlated with GT or DMI. Rumen pools can be accurately predicted under discontinuous feeding regimes, although the representation of rumen ammonia pools requires further development. This general finding is highly relevant since the distance between the "sward-driven" and "metabolic driven" models can be shortened and the whole and unique process of "ingestion-digestion" of nutrients under grazing tackled. Grazing time control remains a difficult obstacle to understand the whole process. This research offered valuable information about the relative importance of several factors in the control of GT. Clearly it is necessary to understand the way in which the different signals produced at different places are integrated for the animal to modulate eating and other behaviour. In this sense the combination of analytical and synthetic research was proven to be an effective strategy.

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STELLINGEN

1. Rumen fill (represented either by dry matter or neutral detergent fibre rumen pool sizes), is unlikely to be the main factor which signals the end of the first grazing session of the day. *This Thesis.*

2. A combination of signals, rather than a single absolute value of any key variable, controls the initiation and termination of individual meals. *This Thesis.*

3. An integration of the unique processes of ingestion and digestion will be required if any progress is to be made in our understanding of feed intake under grazing conditions. *This Thesis*

4. Our lack of understanding about the control of grazing time, is the main obstacle preventing the prediction of dry matter intake under grazing conditions. *This Thesis.*

5. Science is the reconstruction of complexity by an expanding synthesis of freshly demonstrated laws. *Wilson, E.O. On Human Nature. Cambridge MS: Harvard University Press, 1978.*

6. Brief periods of revolutionary science are punctuated by long periods of normal science. *Kuhn, T.S. The Structure of Scientific Revolutions. Chicago, IL: University Press, 1963.*

7. The potential role of mathematics in science and technology must be acknowledged as an integral part of the basic logic underlying these vital disciplines. *Dijkstra, J. and France, J. Modelling and Methodology in Animal Science. In: Proc. IVth Intern. Workshop on Modelling Nutrient Utilisation in Farm Animals. Foulum, Denmark: National Institute of Animal Science, 1995.*

8. Truth comes out of error more easily than out of confusion. *Francis Bacon (1561-1626)*

9. As a supposedly successful species, we seem to be pretty confused.

Stellingen bij het proefschrift
Pablo Chilibroste.
Grazing Time: The Missing Link. A study of the plant-animal interface by integration of experimental and modelling approaches.
Wageningen, 28 September, 1999.

This Thesis is dedicated to my wife

CONTENTS

GENERAL INTRODUCTION

GENERAL INTRODUCTION

On a worldwide scale, a negative correlation exists between average milk price received by the farmers and the proportion of grazed sward. A closer correlation exists between the proportion of grazed area and the relative milk production cost (Clark and Jones, 1995). Market oriented dairy systems as in Uruguay and New Zealand face a permanent constraint in milk production cost of a maximum US$ 0.08 to 0.12 per litre produced. Nevertheless, milk production in Uruguay has been growing at an average annual rate of approximately 7% (DIEA-MGAP, unpublished) in the last two decades. Uruguayan dairy production systems are predominantly based on direct grazing of temperate grasses and legumes (both as monocultures and as mixtures) usually supplemented with conserved forages (maize and pasture silage) and concentrate (pure grains, industrial by-products and/or compound feeds). The use of supplements by dairy farmers has grown as stocking density increased (Duran, 1996). Nevertheless (young growing) pasture (grass, legume or mixtures) with high nutritive value remains the cheaper feed ingredient to feed the cows, and an efficient use of the pasture is essential to minimise the costs of production. Although the cows graze all the year round, herbage growth during winter and autumn is limited and a shortage of available herbage may occur. This problem becomes critical in autumn when a large proportion (> 70 %) of a herd's calvings are planned, a policy stimulated by the higher milk prices paid by the dairy companies in winter. Well-tuned feeding strategies (a combination of grazing and feeding concentrates and roughage) are essential in autumn and winter to be able to exploit animal potential (including pregnancy for the next lactation) on the one hand and to achieve profitability on the other. Besides, the importance of an efficient grazing process in the long-term has recently been emphasised (Parsons and Chapman, 1998). Even though most of the research topics covered in this Thesis apply to the grazing field in a wider sense, the problems outlined above exerted a major influence both in the methodologies utilised, and in the General Discussion.

In grazing systems whether intensive or not, the plant-animal interface is a central feature (Hodgson, 1985, Forbes 1988) and dry matter intake (DMI) is the major determinant of animal production (Forbes 1995; Grovum 1987; Ungar 1996). In intensively managed grazing systems DMI is the most appropriate criterion on which to base tactical or within season decisions (Ungar, 1996) including:

- level and type of supplementation for the dairy herd,
- grazing area allocation,
- grazing system,
- control of grazing time and daily schedule,
- grouping of cows, etc.

A better understanding and predictability of DMI under grazing conditions should result in better management and utilisation of the available resources.

Daily DMI may be seen as the summation of individual discrete meals (Forbes, 1995). Under grazing conditions, periods of eating and fasting alternate. For lactating dairy cows, two major grazing bouts have been observed: one in the morning and the largest in the afternoon (Rook et al., 1994; Gibbs et al., 1997). Under daily strip-grazing, this ingestive behavior may be even more pronounced (Chilibroste et al., unpublished) with a quick depletion of the available

pasture. Understanding which factors control a meal would lead to the understanding of daily DMI (Forbes, 1995), although different factors might operate at different times during the day (Gill and Romney, 1994).

On a daily basis, DMI under grazing is commonly expressed as the product of intake rate (IR) and grazing time (GT). In recent decades substantial progress has been made in the understanding and quantification of the main mechanisms determining IR (see recent reviews of Demment et al., 1995; Illius and Hodgson, 1996; Parsons and Chapman, 1998; Ungar, 1996). The bite has been suggested as the functional link between the spatial and morphological properties of the vegetation and the ingestive apparatus of the animal (Laca et al., 1992; Laca et al., 1994a), and has the largest influence on IR (Ungar, 1996). Parsons and Chapman (1998) summarize this achievement with an illustrative sentence "...although humans regard utilisation as a problem to be solved on a paddock by paddock basis, grazing animals are constrained to meeting their dietary requirements bite by bite.". Figure 1 shows conceptual diagram of the components of ingestive behavior that mediate between sward structure and short-term intake rate (Ungar, 1996).

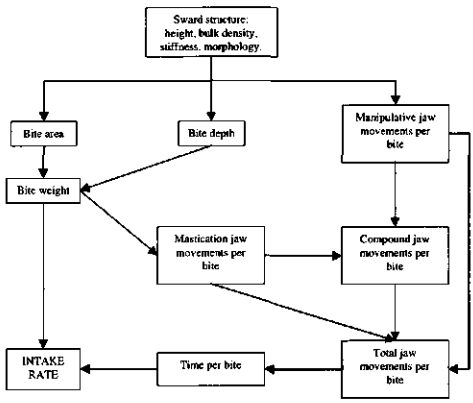

Figure 1. The components of ingestive behavior, which mediate between sward structure and short-term intake, rate. Adapted from Ungar, 1996.

The basic relationships shown in Figure 1 have been developed at feeding station level but has been used as a theoretical background for a larger scale research. They have also been integrated into dynamic simulation models (e.g. Demment et al., 1995; Newman et al., 1995). Despite the fact that the conceptual relationships outlined in Figure 1 are tightly established, the quantitative relationships are less certain since changes in spatial scale (Laca et al., 1994b; Ungar, 1996) and animal state (Greenwood and Demment, 1988; Newman et al., 1995) influence short term intake rate.

In contrast with the progress made to understand control IR, little progress has been made in the understanding of control of GT. This gap has been clearly stated by authorities in the grazing field: "...there has been little progress in providing a mechanistic account of the factors controlling grazing time, even though this is essential to progress from an understanding of instantaneous intake rate to understanding daily intake." (Parsons et al., 1994), "...there is currently no known physiological mechanism that adequately explains grazing time." (Newman et al., 1995), "...prediction of grazing time has been a fundamental but elusive goal of intake models." (Laca and Demment, 1996). It contrasts with the large body of research carried out to understand the ultimate control of individual meals in housed animals. Rumen fill, rumen concentration of fermentation products, balance of nutrients, and concentration of metabolites and hormones in blood have been identified as the main candidates controlling DMI (e.g. Faverdin et al., 1995; Forbes, 1995; Grovum, 1987; 1995; Ketelaars and Tolkamp, 1991; Leuvenink, 1998). Due to the high cell wall concentration in grass, rumen fill has been also pointed as controlling DMI under grazing conditions (Cherney and Mertens, 1998). However, Van Vuuren (1993) rejected rumen fill as an important factor and suggested that the concentration of fermentation products controlled DMI in cows fed fresh ryegrass. In addition, Tamminga and Van Vuuren (1996) speculated that total chewing time could be as a possible constraint to DMI for low-DM-forage fed dairy cows which agrees with the findings of John and Ulyatt (1987) in sheep.

To integrate intake on a daily basis, a translations has to made from IR while actively grazing to daily DMI where periods of eating and other important activities like rumination, idling, resting, etc, are alternated (Demment et al., 1995). Management decisions might be taken in daily or larger scale, but the underlying processes take place continuously (digestion) or in discrete bouts (ingestion) within a day. The integration of digestive and ingestive processes under grazing is required to understand and eventually predict nutrient supply to the grazing animal supplemented or not with extra feed. The modelling of rumen fermentation and supply of nutrients to the ruminant has advanced greatly in recent decades (Dijkstra and France, 1996). However, few attempts have been made to work under non-steady state conditions (Newman et al., 1995), a situation which seems to be more appropriate to model DMI under grazing conditions.

OUTLINE OF THIS THESIS

The main objectives of this Thesis are:

1. To gain insight into the main mechanisms controlling DMI, IR and GT during the first grazing session after a.m. milking.
   Effects of the length of grazing session, the combination of starvation and rumen fill before grazing, and the sward characteristics on ingestive behaviour (bite mass and bite rate), IR and GT were studied (Chapters 2, 3, 5 and 6).

2. To judge the relative importance of rumen fill and the concentration of fermentation products in the rumen liquor as candidates to signal the end of the grazing sessions (Chapters 2, 3, 5 and 6).

3. To evaluate the use of the gas production technique as an alternative *in-vitro* method to characterise rumen content fermentability in animals exposed to different treatments involving periods of starvation, rumen fill and allowed grazing time (Chapter 4).

4. To develop (Chapter 1) and design and evaluate (Chapter 7) simulation models operating under non-steady state conditions aimed either to predict DMI (Chapter 1) or to predict rumen fermentation and supply of nutrients (Chapter 7).

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1 NUTRITIONAL EVALUATION OF DIETS. SIMULATION MODEL OF DIGESTION AND PASSAGE OF NUTRIENTS THROUGH THE RUMEN-RETICULUM

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NUTRITIONAL EVALUATION OF DIETS. SIMULATION MODEL OF DIGESTION AND PASSAGE OF NUTRIENTS THROUGH THE RUMEN-RETICULUM

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ABSTRACT

To evaluate the nutritional behaviour of simple and complex diets provided under different feeding regimes, a stochastic, dynamic and predictive simulation model was developed. Feed fractions, considered in the model were soluble non-structural carbohydrates, insoluble non-structural carbohydrates (INSC), INSC degradation rate (kd_{INSC}), fractional passage rate (kp), neutral detergent fibre (NDF), NDF potentially digestible (PNDF), PNDF degradation rate (kd_{PNDF}), soluble crude protein (SCP), insoluble crude protein (ICP), ICP potentially degradable (PICP), PICP degradation rate (kd_{PICP}). Animals characteristics considered in the model, were live weight and physiological status (growth, fattening, lactation and/or pregnancy). Variables inherent to management practices were amounts and schedule of DM offered to the animals. The model was subjected to validation for a wide range of experimental conditions. Predictions of total DM and forage DM intakes (and therefore the estimate of the substitution of forage for concentrate) had an $R^2 = 0.95$ and 0.91 respectively. Prediction of NDF digestibility had an $R^2 = 0.61$ in a smaller range of experimental conditions.

Keyword: simulation model, rumen, intake.

INTRODUCTION

In production systems, where feeding of animals is through grazing as the only source of nutrients or complemented with other feed sources such as conserved forages and/or concentrates, the feeding regime is discontinuous throughout the day. Interactions between the different feed and/or individual nutrients are functions of the nutritional characteristics of the feedstuff, the sequence and level of feeding, the animal potential and the environment (Chilibroste, 1992). These characteristics make such production systems, predominantly present in the southern hemisphere, different from those of the northern hemisphere, where the majority of the scientific information has originated. This means that many of the technologies developed to improve the efficiency of high production systems under confinement are not necessarily applicable to grazing systems. There is abundant work that has studied factors affecting voluntary intake in ruminant animals, mostly under confinement conditions (Conrad, 1966; Freer, 1981; Hodgson, 1982; Minson, 1982; 1990). In forage based animal production systems a high correlation has been demonstrated between animal response and voluntary dry matter intake (Minson, 1990). Therefore, having the capacity to predict animal voluntary intake and the level of nutrient extraction from consumed feeds, has a strategic value in management practices for such systems as well as for the analysis of improvement alternatives.

In ruminant animals the amount and type of nutrients available for absorption and metabolism, differs profoundly from the profile of nutrients present in the feed consumed, and these

differences primarily result, from rumen microbial activity (Russell et al., 1992; Dijkstra, 1993). Maximal ruminal fermentation is characterized by high dry matter digestion together with an optimum level of microbial efficiency, goals that seem to depend on the quantity and ratio between the energy and protein supplied by the diet (Stokes et al., 1991b).
The reported mathematical and/or simulation models (Conrad et al., 1964; Forbes, 1977; Mertens and Ely, 1979; ARC, 1980; Mertens and Ely, 1982; Bywater, 1984; France et al., 1982; Kahn and Spedding, 1983; Jarrige et al., 1986; Baldwin et al., 1987; Fisher et al., 1987; Mertens, 1987; NRC, 1988; Ørskov et al., 1988b; Doyle et al., 1989; Hyer et al., 1991a,b; Seman et al., 1991; Aguilar and Cañas, 1992; Sniffen et al., 1992; Dijkstra, 1993), present great variability in the proposed objectives, although most of their titles directly reference dry matter intake (Chilibroste, 1993). The approach has been predominantly energetic, with few cases where energy and protein requirements are considered as a whole (ARC, 1980; NRC, 1988), and only the most recent studies take into account energy-protein interactions (Russell et al., 1992; Sniffen et al., 1992; Dijkstra, 1993). Previously mentioned models mostly work on the hypothesis of continuous supply of substrate. In general, these models evaluate one feed at a time (individual feed as well as complete diets) and little attention has been given to the construction of models with a combination of two or more feed ingredients fed separately, and the positive or negative associative effects derived from them.
The objective of the present study is to evaluate the nutritional behaviour of simple or complex diets under different nutritional regimes. To met this goal a simulation model, integrating and quantifying the dynamics of the simultaneous processes of digestion and passage of the nutrients through the reticulo-rumen was developed. The hypothesis to be tested is that when voluntary intake in ruminants is controlled by physical distension, the dry matter intake and ruminal digestion of the main nutrients can be predicted from quantification of the simultaneous processes of digestion and passage of nutrients through the rumen.

METHODOLOGY

A stochastic, dynamic (France and Thornley, 1984) and predictive simulation model was developed in the GW.Basic language. The objective of the model is not to describe the process as it occurs naturally, dividing it in as many components as the available knowledge allows it, but to represent the function of the system, resulting in an acceptable predictive capacity for a wide range of production situations. A list of symbols and terms referred in text are shown in Table 1.

Model structure

The general structure of the model and the feed fractions dynamics are presented in Figure 1 and 2, respectively. The model allows the simultaneous use of up to 5 different feeds with a preferential intake of concentrates over forages and of these over silage. In the models conception and construction, it was assumed that the animal has a potential maximum neutral detergent fibre rumen (NDF) capacity (MRC_{NDF}, Figure 1) which varies along its life cycles, and that the feeds have a given capacity to occupy space determined by its NDF content.

Table 1. List of abbreviation used in the description of the model

Symbol	Description	Unit
NSC	Non-structural carbohydrates	% DM
SNSC	Water soluble non-structural carbohydrates	% DM
INSC	Water insoluble non-structural carbohydrates	% DM
NDF	Neutral detergent fibre	% DM
PNDF	Fraction of NDF potentiality digestible	% NDF
UNDF	Undegradable NDF	% NDF
CP	Crude protein	% DM
SCP	Water soluble crude protein	% CP
ICP	Water insoluble crude protein	% CP
PICP	Water insoluble crude protein potentiality digestible	% ICP
DCP	Degradable crude protein: SCP + PICP	% DM
UICP	Undegradable ICP	% ICP
kp	Potential fractional passage rate of insoluble fractions	%/h
kpe	Effective fractional passage rate of insoluble fractions	%/h
$kd_{(i)}$	Potential fractional degradation rate of fraction sub i	%/h
kde	Effective fractional degradation rate of fraction sub i	%/h
MRC_{NDF}	Maximal NDF rumen capacity	% of BW
ARC_{NDF}	Actual NDF rumen capacity	% of BW

BW= body weight

Besides, feed disappearance from the rumen is given by two simultaneous and competitive processes: degradation and passage, both represented by a first order kinetics. Degradation occurs at a certain rate (kde_i, Figure 1) varying with ruminal availability of non-structural carbohydrates (NSC) and potentially degradable protein (DCP) in each hour, thus considering the effect of the ruminal environment upon microbial activity. These parameters (availability of NSC and DCP) are function of the type of feed consumed and the feeding regime. The effective rate of passage (Kpe, Figure 1) of the insoluble solid fractions is determined by the extension of NDF rumen fill. Hour was defined as the basic time unit in which the model operates. At each hourly model iteration, DM intake is calculated as the difference between MRC_{NDF} and the actual NDF rumen capacity (ARC_{NDF}) divided by the NDF content of the available feed (provided that ARC_{NDF} # than 80 % of MRC_{NDF}. The model iterates during 24 hours a day for 8 consecutive days, giving results for the 8th day when an equilibrium has been reached.

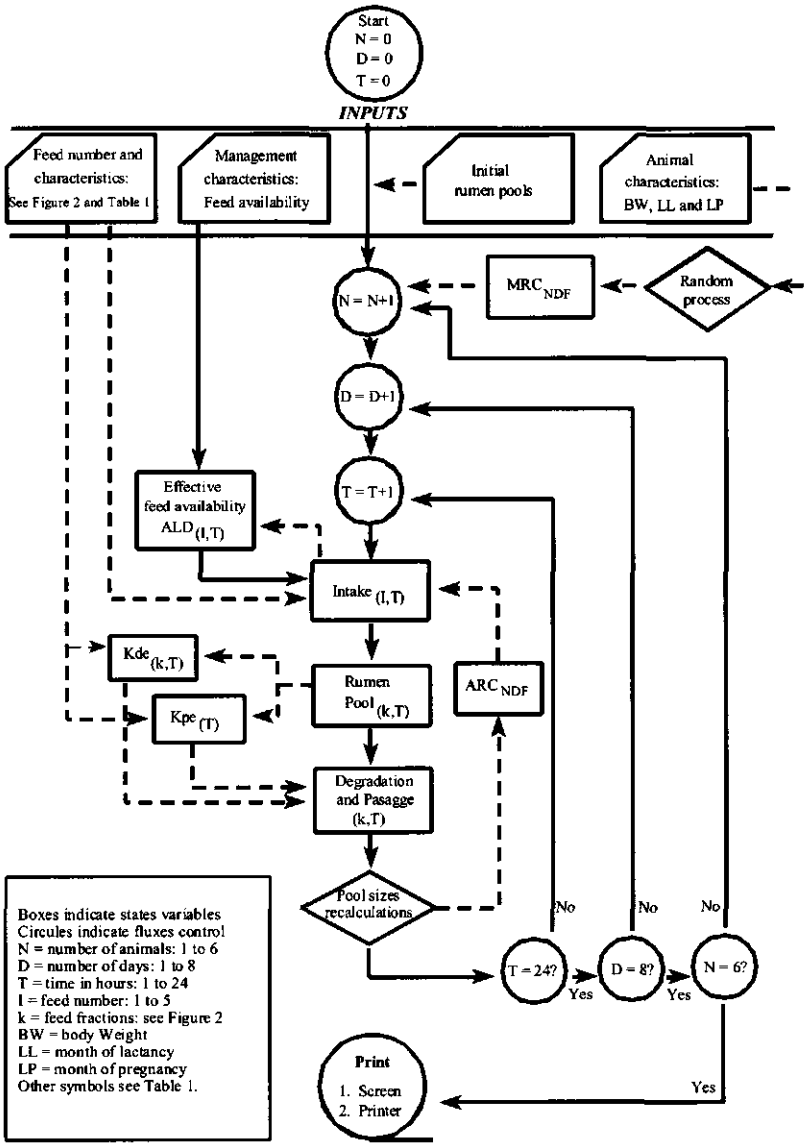


Fig. 1. Model structure and operation.

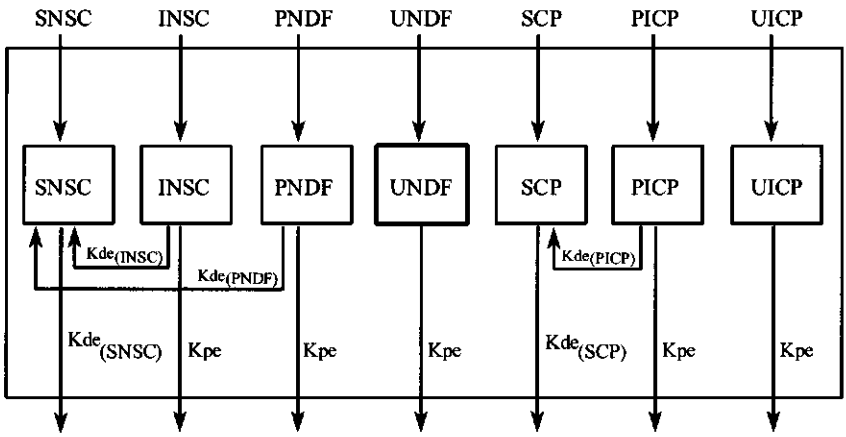


Fig. 2. Feed fractions ruminal dynamics.

Model components and exogenous variables.

Feed inputs

The required feed inputs are SNSC, INSC, NDF, PNDF, CP, SCP, ICP, PICP (Table 1). Carbohydrate characterization (structural and non-structural) for high production cows, has become important only in recent years. The interest for a better characterization of the different fractions is due to the fact that carbohydrates are the most important energy source for the rumen microflora and that fermentation differs largely depending upon the feed sources (Nocek and Tamminga, 1991). Non-structural carbohydrates are composed by the carbohydrates not recovered in the NDF fraction, including sugars, starch, fructans, galactans, pectines, β-glucans, etc. (Van Soest et al., 1991). This group of carbohydrates are active components of the plant metabolism and have a rapid and total fermentation potential within the rumen. Although important advances have been achieved in the knowledge and quantification of the ruminal fermentation process in recent years, the exact quantities of carbohydrates and protein required at the ruminal level to maximize microbial performance is not yet known (Aldrich et al., 1993). While soluble feed components occupy little space in the rumen, fibre displaces considerable volume and NDF is the only chemical method isolating all the fibre components of the feed (cellulose, hemicellulose, and lignin) (Mertens, 1987). Van Soest et al., (1991), established that NDF is more closely related to the daily ruminating time, gastrointestinal tract filling and dry matter intake, than other fractions such as crude fibre and acid detergent fibre. Thus, space occupying capacity of each feed was assigned in terms of their NDF content.

As for carbohydrates, N was differentiated between soluble fractions rapidly available for microorganisms, the potentially degradable insoluble fraction which has a slower availability and the indigestible fractions linked to the matrix of the cell wall which is not available for ruminal fermentation. The rumen indigestible fractions disappear only through passage (Figure 2). The advantages of using this classification of N, over one applying only chemical and/or biochemical criteria, has been widely documented (Van Soest, 1982; Kristensen et al., 1982; Lindberg, 1985; NRC, 1985; Nocek and Russell, 1988; Ørskov, 1988c; Sniffen et al., 1992).

In the proposed model, degradation and passage of the different fractions was assumed to be described by as first order kinetics (Robinson et al., 1986). As inputs the model require potential degradation values for carbohydrates as well as for the N fractions. Although the effect of ruminal conditions on microbial activity and on degradation of insoluble fractions is well known (Hoover, 1986; Hoover and Stokes, 1991), it has usually not been incorporated in ruminal fermentation models. The models reported by Argyle and Baldwin (1988) referred by Dijkstra (1993), Russell et al. (1992) and Dijkstra (1993) are the first that consider the negative effect of low ruminal pH on NDF degradation. In the present model the effective degradation rates (Kde, Figure 1) of INSC, PNDF and PICP are estimated each hour according to the ruminal availability of NSC and DCP at that moment. The potential degradation rate of NSC at high ruminal levels of NSC, is only affected by the availability of N given the high tolerance of amylolytic microorganisms to low pH levels (Hoover and Stokes, 1991). Reduction factors of the potential degradation rates used in the model are presented in Table 2. The reduction factors proposed accommodates the concept of differential capacity of cellulolitic and amylolytic microorganisms to take up N and the increased requirements for ruminal degradable protein, if the NSC availability rises (Ørskov, 1988c; Stokes et al., 1991a,b). The fixation of these levels is an arbitrary and preliminary approximation while more precise information is generated.

The dynamic nature of the digestion process indicates that the extension of digestion is a function of time feed remains in the gastrointestinal tract. In our model is assumed that each feed has its specific passage rate and this value must be provided assuming ad-libitum DM intake. The effective fractional passage rate (kpe, Figure 1) of the different insoluble fractions is calculated at each model iteration as function of the actual NDF rumen capacity (ARC_{NDF}) expressed as fraction of MRC_{NDF}. For calculations an arbitrary scale was defined (Table 2), using the values reported by Sniffen et al. (1992) as a reference.

Table 2. Calculation of effective fractional rates

Degradation rates		
Ruminal availability as % of rumen DM content		Calculation of reduction factors
NSC	DCP	
NSC # 5	DCP □ 6	$Y_1= Y_2= Y_3= 0.7$
	DCP # 6	$Y_1= Y_2= Y_3= 0.11*DCP$
5<NSC # 8	DCP □ 8	$Y_1= Y_2= Y_3= 0.75$
	DCP # 8	$Y_1= Y_2= Y_3= 0.0937*DCP$
8<NSC # 10	DCP □ 10	$Y_1= Y_2= Y_3= 0.85$
	DCP # 10	$Y_1= Y_2= Y_3= 0.085*DCP$
10<NSC # 15	DCP □ 10	$Y_1= Y_2= Y_3= 0.9$
	DCP # 10	$Y_1= Y_2= Y_3= 0.09*DCP$
15<NSC # 35	DCP □ 12	$Y_1= Y_2= Y_3= 1.0$
	DCP # 12	$Y_1= Y_2= Y_3= 0.083*DCP$
35<NSC # 40	DCP □ 15	$Y_1= 1.0$; $Y_2= Y_3= 0.9$
	DCP # 15	$Y_1= 0.083*DCP$; $Y_2= Y_3= 0.075*DCP$
40<NSC # 50	DCP □ 15	$Y_1= 1.0$; $Y_2= Y_3= 0.7$
	DCP □ 12	$Y_1= 0.066*DCP$; $Y_2=Y_3= 0.07*DCP$
	DCP # 12	$Y_1= 0.066*DCP$; $Y_2=Y_3= 0.058*DCP$
NSC > 50	DCP □ 15	$Y_1= 1.0$; $Y_2= 0.4$; $Y_3= 0.5$
	DCP □ 12	$Y_1= 0.066*DCP$; $Y_2=0.4$; $Y_3=0.5$
	DCP # 12	$Y_1= 0.066*DCP$; $Y_2=0.033*DCP$; $Y_3= 0.0416*DCP$
Passage rates		
Fill as $(ARC_{NDF}/MRC_{NDF})*100$		
65<FILL #85		Kep = Kp * 0.85
45<FILL #65		Kep = Kp * 0.65
FILL #45		Kep = Kp * 0.55

Y_1, Y_2 and Y_3= reduction factors applied over Kd_{INSC}, Kd_{NDF} and Kd_{ICP} respectively; other symbols see Table 1.

Animal input variables

Because the space occupying capacity of feeds was expressed in terms of NDF content, the host capacity of the animal or ruminal volume, should be expressed in the same units. Since ruminal volume is a function of body weight (Van Soest, 1982), this variable is expressed in the model in terms of kg of NDF as percentage of the body weight. According to Visser De et al. (1992; 1993)

and Bosch et al. (1992a) in which determination of rumen pool sizes were done, a value of 0.9% of body weight in terms of NDF (kg), was chosen as MRC_{NDF}. This value is corrected by animal physiological status (lactation and/or pregnancy) according to ARC (1980).
Taking in account the high individual variability that the animals present in terms of voluntary intake (Hartnell and Satter, 1979; Ørskov et al., 1988a), the MRC_{NDF} used during the simulation is estimated as a random variable according to Naylor et al. (1966) as follows:

$$x = \sigma_x * (12/K)^2 * (3r_i-(K/2)) + u_x$$

where: x = normally distributed random variable; u_x and σ_x^2 = mean and variance, respectively, 3 sum from i = 1 to K and K = sum of 24 random numbers defined over the interval 0 to 1.

Management inherent variables

The schedules and amount of dry matter offered to the animals from each feed are inputs to the model. Additionally, restriction periods can be defined during the day, this means the hours in which the animal has no access to one or various of the offered feeds thus, simulating enclosure, controlled grazing or milking time.
This model structure allows simulation of discontinuous feeding conditions and other situations in which feeds with different characteristics are offered at different times throughout the day.

Model State variables

Actual NDF rumen capacity

ARC_{NDF} (Figure 1) is calculated each hour, and it is an indicator of the available space in the rumen for newly ingested feed. It is calculated as MRC_{NDF} minus the amount of NDF that remains in the rumen at each iteration.

Effective availability of feed

The amount of feed effectively available to the animal at a given hour "t" (ALD(i,t), Figure 1), is equal to the feed availability in the previous hour (t-1), minus the feed intake in the previous hour. The availability is equal to 0 during a restrictive period (animal has no access to the feed) and is equal to the amount of feed offer, during the hour when the feed is offered to the animals.

Pools

The model accounts for the state of seven pools (Figure 2) where the contribution of each feed is kept. The pools are expressed in kg. The dynamic of the different fractions in the rumen is defined by equations 1 to 7 as follows:

$$\boldsymbol{P}SNSC_{(I,T)} = DMI_{(I,T)} * SNSC_{(I)} + \boldsymbol{P}INSC * Kde_{(INSC)} + \boldsymbol{P}PNDF_{(I,T)} * Kde_{(PNDF)} \quad (1)$$

$$\boldsymbol{P}INSC_{(I,T)} = DMI_{(I,T)} * INSC_{(I)} + \boldsymbol{P}INSC_{(I,T-1)} - \boldsymbol{P}INSC_{(I,T)} * Kde_{(INSC)} - \boldsymbol{P}INSC_{(I,T)} * Kpe \quad (2)$$

$$\boldsymbol{P}PNDF_{(I,T)} = DMI_{(I,T)} * NDF_{(I)} * PNDF_{(I)} + \boldsymbol{P}PNDF_{(I,T-1)} - \boldsymbol{P}PNDF_{(I,T)} * Kde_{(PNDF)} - \boldsymbol{P}PNDF_{(I,T)} * Kpe \quad (3)$$

$$\boldsymbol{P}UNDF_{(I,T)} = DMI_{(I,T)} * NDF_{(I)} * (1-PNDF_{(I)}) + \boldsymbol{P}UNDF_{(I,T-1)} - \boldsymbol{P}UNDF_{(I,T)} * Kpe \quad (4)$$

$$\boldsymbol{P}SCP_{(I,T)} = DMI_{(I,T)} * CP_{(I)} * SCP_{(I)} + (\boldsymbol{P}ICP * Kde_{(ICP)}) \quad (5)$$

$$\boldsymbol{P}PICP_{(I,T)} = DMI_{(I,T)} * CP_{(I)} * PICP_{(I)} + \boldsymbol{P}PICP_{(I,T-1)} - \boldsymbol{P}PICP_{(I,T)} * Kde_{(PICP)} - \boldsymbol{P}PICP_{(I,T)} * Kpe \quad (6)$$

$$\boldsymbol{P}UICP_{(I,T)} = DMI_{(I,T)} * CP_{(I)} * (1-PICP_{(I)}) + \boldsymbol{P}UICP_{(I,T-1)} - \boldsymbol{P}UICP_{(I,T)} * Kpe \quad (7)$$

where: i = feed 1 to 5; T = hour 1 to 24; ***P*** state for pool; other symbols see Table 1.

MODEL EVALUATION

According to Black et al. (1993), validation is determined by the precision and level of certainty that a model achieves in its prediction under a wide range of simulation conditions. The wider the range of experimental and/or productive situations in which the model predicts accurately, the more reliable the concepts and parameters with those it was built and its predictions, will be. In this study the simulation results were compared with results of controlled experiments by a T-test (Steel and Torrie, 1980) and a regression line between the observed and predicted results was build. A detailed description of the experiments used to validate the model is shown on Tables 3, 4 and 5, respectively.

Table 3. Description of experiments used for validation

Ref.	Author	Animal breed	Weigh (kg)	Treatments
1.	Hartnell and Satter (1979)	Holstein	606 628 656 700	T1= H+G (45:55) early lactation T2= H+G (57:43) mean lactation T3= H+G (67:33) late lactation T4= H+G (82:17) dry period
2.	Bosch et al., (1992)	Holstein-Frisian	546 569 550 576 560 579	T1= G1 + 1 kg conc., late lactation T2= G1 + 7 kg conc., early lactation T3= G2 + 1 kg conc., late lactation T4= G2 + 7 kg conc., early lactation T5= G3 + 1 kg conc., late lactation T6= G3 + 7 kg conc., early lactation
3.	Elizalde et al.,	Holstein-A. Angus	615	T1= Grazing oats; Period 1 T2= T1 + silage maize. T3= Grazing oats; Period 2 T4= T3 + silage maize.
4.	Fredrickson	Hereford-A.Angus	194 194 194 194	T1= Hay ad. Libitum T2= T1 + 0.9 kg barley grain T3= T1 + 0.85 kg corn grain T4= T1 + 0.89 kg wheat grain

Ref.=reference; H+G= hay plus grain (forage:concentrate relationship); conc.= concentrate; G1,G2,G3= early, mead and late silage harvested time respectively; T1,2,..= treatment number.

Table 4. Description of feeds used during validation: crude protein

Ref.	Feed name	DM %	CP %DM	SCP %CP	ICP %CP	PICP %ICP	Kd_{PICP} %/h
1	Hay	89.8	17.2	28.0	72.0	60.0	8.5
	Grain	90.1	18.3	30.0	70.0	75.0	8.0
2	Silage G1	59.4	21.3	58.0	42.0	93.0	12.0
	Silage G2	54.3	19.6	58.0	42.0	93.0	12.0
	Silage G3	60.8	20.9	58.0	42.0	93.3	12.0
	Concentrate	88.1	18.2	40.0	60.0	80.0	10.0
3	Oat. Per 1.	22.6	14.3	45.0	55.0	80.0	5.0
	Oat. Per 2.	22.8	12.0	30.0	71.0	71.0	5.0
	Silage Maize 1.	27.0	8.2	67.0	33.0	85.0	4.0
	Silage Maize 2.	27.6	8.5	60.0	40.0	85.0	4.0
4	Hay	s/d	8.9	25.0	75.0	75.0	10.0
	Barley	s/d	13.8	25.0	75.0	60.0	12.5
	Maize	s/d	13.4	15.0	85.0	76.0	3.5
	Wheat	s/d	13.3	30.0	70.0	75.0	8.0

M1, M2= silage maize period one and two respectively; Other symbols see Table 1 and 3.

Table 5. Description of feeds used during validation: carbohydrates

Ref.	Feed name	SNSC %DM	INSC %DM	Kd_{INSC} %/h	NDF %DM	PNDF %NDF	Kd_{PNDF} %/h	Kp %/h
1	Hay	10.0	15.0	30.0	49.4	70.0	4.0	3.5
	Grain	21.6	32.5	15.0	17.5	80.0	4.5	4.5
2	Silage G1	12.8	8.1	15.0	44.6	89.0	6.0	3.1
	Silage G2	10.8	5.6	15.0	54.7	82.0	6.0	3.7
	Silage G3	10.3	3.7	15.0	54.8	84.0	6.0	4.4
	Concentrate	26.2	17.5	15.0	28.6	85.0	6.0	4.0
3	Oat. Per 1.	10.0	16.0	25.0	38.2	78.0	6.0	3.5
	Oat. Per 2.	10.0	16.0	25.0	42.4	70.0	6.0	3.5
	Silage M 1.	10.0	10.0	25.0	47.8	50.0	4.0	3.0
	Silage M 2.	10.0	10.0	25.0	54.1	50.0	4.0	3.0
4	Hay	5.0	5.0	30.0	63.2	70.0	6.0	3.5
	Barley	38.9	21.4	24.2	17.3	90.0	14.5	4.0
	Maize	20.2	53.2	4.0	11.3	90.0	5.1	5.0
	Wheat	47.5	21.2	18.2	11.4	70.0	15.0	4.0

For symbols see Table 1.

In all the experiments used for validation, the information of the chemical composition of the feed corresponds to the one reported by the authors. The feed potential values for degradation parameters (digestion rate and extension) and passage rate, were estimated from different sources:

1. In Hartnell and Satter's study (1979) the protein and structural carbohydrate degradation parameters were estimated from Nocek and Russell (1988) and Madsen and Hvelplund (1985), while the ruminal passage values were estimated from Sniffen et al. (1992).

2. In Fredrickson et al.'s study (1993), the degradation and passage parameters of hay were taken from Sniffen et al. (1992) and the parameters for grains were estimated from the information reported by Tamminga et al. (1990).

3. In Bosch et al.'s study (1992a,b) the degradation values of silage CP (rate and extension) were estimated from Sniffen et al. (1992), while the potential degradation and passage values of NDF and particulate fractions were taken from the study itself. The values assigned to the concentrate were built, based on the composition of the mixture and degradation values reported by Tamminga et al. (1990).

4. In Elizalde et al.'s study (1992) passage rates values were taken from Sniffen et al. (1992), while the potential degradation values (CP and NDF) were taken from the ones reported in the study itself.

The comparison of results reported in the experiments with the ones obtained in the simulation, given the experimental conditions described on Tables 3, 4 and 5, are shown in Table 6. In addition, Figures 3 and 4, show the regression coefficients between the observed and model predicted values, for total DM and forage intake, respectively. The values of Elizalde et al.'s study (1992) which appear on figure 4 correspond to oat and corn silage intake.

The correlation between the simulated and observed values (R^2 = 0.95 and R^2 = 0.92 for total DM and forage intake, respectively) was high. For total DM intake, 14 of the 18 treatments used in the validation (Table 6) were statistically similar while 4 differed ($p<0.05$). Three of the latter 4 treatments, correspond to feeds with a lower NDF and higher CP and NSC content (Table 4 and 5). It is possible that in these conditions the intake regulation was not determined by animal rumen fill, thus explaining the tendency of the model to overestimate the value of total DM and forage intake. Gasa et al. (1991), while working with Lolium perenne silage, with similar chemical composition to the G1 silage use by Bosch et al. (1992a) (Ref.2, Tables 4 and 5), and with similar supplementation levels, determined that intake regulation with those materials was not caused by fill. The lower NDF pools measured by Bosch et al. (1992a) for this silage (G1) at low or high levels of concentrate supplementation (4.7 and 5.4 kg NDF respectively) compared with silage G2 (5.9 and 7.4 kg NDF) can be considered additional evidence in this respect. The particulate fractions passage rate values obtained in the experiments and estimated by the model were similar for Bosch et al. (1992b) (3.14 vs 3.00 % /h respectively for T1) and higher in the case of Elizalde et al. (1992) (4.0 vs 3.3 %/h respectively for T1).

Table 6. Experimental and model results comparison. Total d y matter and forage intake.

Ref.	Treatment	DM Total		DM Forage	
		Observed kg/day	Simulated kg/day	Observed kg/day	simulated kg/day
1.	T1	16.9	17.6 (0.87)		
	T2	19.8	19.1 (1.27)		
	T3	17.6	16.9 (1.02)		
	T4	10.8	11.2 (1.17)		
2.	T1	12.8	16.1**(1.37)	11.8	
	15.1**(1.37)				
	T2	17.0	19.9**(1.32)	9.7	
	13.0**(1.32)				
	T3	12.0	11.9 (1.28)	11.0	10.9 (1.28)
	T4	17.0	16.7 (0.60)	9.7	10.0 (0.60)
	T5	15.6	15.0 (1.64)	14.0	14.6 (1.64)
	T6	19.0	18.2 (0.95)	11.2	12.0 (0.95)
3.	T1	14.7	17.5**(0.61)		
	T2	12.8	12.9 (0.63)	6.9(Oat)	7.7 (0.17)
				5.9(S.M)	5.2 (0.35)
	T3	13.6	14.5 (0.76)		
	T4	12.2	12.3 (0.32)	8.1(Oat)	7.6 (0.17)
				4.1(S.M)	4.7 (0.17)
4.	T1	3.3	3.2 (0.17)	3.3	3.2 (0.17)
	T2	4.2	3.9 (0.47)	3.3	3.0 (0.47)
	T3	4.2	3.8**(0.2)	3.4	3.0**(0.2)
	T4	4.1	3.9(0.3)	3.2	3.1 (0.3)

kg/day= kilogram per day; S.M= silage maize; **= ($p<0.05$); values between brackets= standard deviation. Other symbols see Table 3.

For forage DM intake, in 11 of the 14 treatments, estimated values by the model and those observed in the experiment did not differ. This means that the model was useful to estimate the substitution of forage by concentrates.

Elizalde et al. (1992) completed estimates of ruminal CP and NDF digestibility. To compare the NDF ruminal degradability estimated by the model with the values reported by Bosch et al. experiment (1992a), the same quantitative procedure than Elizalde et al. (1992) was used. In Table 7 is shown the comparison between ruminal digestibility of NDF and CP obtained by Elizalde et al. (1992) and of NDF estimated from Bosch et al.'s work (1992a,b), with the resulted degradation values from the simulation model. The regression of simulated values over observed values gave an $R^2 = 0.61$ and a $\beta 1 = 0.974$ not significantly different from 1 ($p<0.05$). Comparison with Bosch et al. (1992a,b) shows that ruminal NDF digestibility

Fig. 3. Total dry matter intake

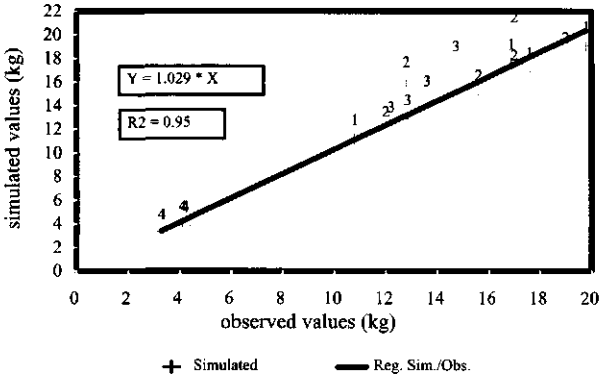
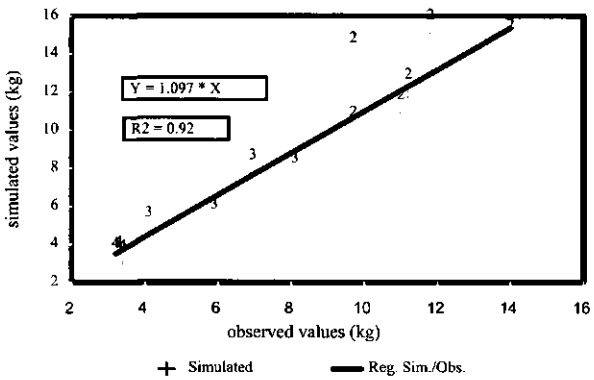


Fig. 4. Foraje dry matter intake



estimated by the model and those estimated from the experimental information, were not different for silage G1 and G3 and significantly different for silage G2 (Table 7). This is not surprising because in the model similar values were included for G2 and G3, due to the similarity of their chemical composition (Tables 4 and 5) and the potential degradation values of NDF (83.4 and 84% for G2 and G3 respectively). The degradability values resulting from the simulation (Table 7, Ref.3) were not different when compared with experimental results for oat CP (T1 and T3), for oat and corn silage NDF, and were significantly higher for silage maize CP ruminal degradation in periods 1 and 2. Lack of agreement between the observed and simulated values for silage CP degradation can be associated as much as to the methods used in the experiment for determination and estimation of degradation parameters (Kristensen et al., 1982; Lindberg, 1985; Madsen and Hvelplund, 1985) or in the utilization of chromium mordant NDF

as a marker in the fractional passage rate estimation (Colucci et al., 1990; Bosch et al., 1992b), as in the assumptions made in the model building. The effective degradation rates estimated by the model during the calculating process, were 2.7 and 2.4%/hour for periods 1 and 2 respectively, which were very similar to the ones estimated in the experiment (2.4%/hour for both periods). Therefore, differences between the observed and predicted values, could have their origins in both, the differences in the assigned values to the potentially digestible fraction of the insoluble CP (85% was assumed in the model) and in the particulate fraction passage rate values.

Table 7. Experimental and model results comparison. NDF and CP ruminal degradability.

Ref.	Tre.	Feed	Ruminal Degradability			
			CP		NDF	
			Observed (%)	Simulated (%)	Observed (%)	Simulated (%)
2.	T1	G1			53.4	55.3 (3.1)
	T3	G2			33.6	49.6**(3.0)
	T5	G3			41.2	42.1 (2.9)
3.	T1	Oat	79.1	83.6 (5.7)	45.9	44.2 (12.7)
	T2	Oat	75.3	79.6**(1.8)	44.0	39.6 (8.4)
	P1	Oat	80.6	85.2 (4.4)	51.6	45.2 (8.4)
	P2	Oat	72.8	77.9 (3.1)	36.6	38.6 (7.5)
	P1	S.M	82.5	94.0**(2.8)	24.6	23.9 (3.8)
	P2	S.M	77.1	88.9**(1.2)	25.9	22.8 (2.0)

For symbols see Table 3.

Intermediate variables calculated by the model, such as ruminal degradation of individual fractions of each feed, variation in the pool size of different fractions throughout the day, and relationships between potential and effective rates of degradation and passage, can not be statistically contrasted with experimental information.

This model identify as a major limitation the lack of reliable information about much of the ingredients commonly used in livestock feeding. Some efforts have been made to establish a data base which include degradation characteristics (rate and extent of degradation) of structural, non-structural carbohydrates and N fractions. However, the lack of a standardized methodology has been the critical point for the development of this area (Nocek and Tamminga, 1991). During the last years efforts have been made in Latin America to standardize the techniques used in feed characterization, making available the information generated in the different regions (RISPAL, 1990).

CONCLUSIONS

When the regulation of the animals voluntary DM intake is controlled by rumen fill (physical regulation), processes that occur at the ruminal level are the most important in determining the efficiency by which the animal uses the feed consumed, and it is at this level, where the majority of the interactions between feed and its fractions are resolved. It is possible to predict the voluntary DM intake and ruminal digestion of the major nutrients, from quantitative integration of the digestion process and rumen feed passage.

From the comparison of the experimental and simulated results (Tables 6 and 7; Figures 3 and 4), it can be concluded that for a wide range of experimental conditions, the model showed a good accuracy level in the prediction of the total DM and forage intake (R^2 = 0.95 and R^2 = 0.92, respectively). The accuracy obtained was lower in the prediction of the NDF ruminal degradation (R^2 = 0.61) and moderate for CP degradation values.

Nutritionally important characteristics of the feed, are: a) the ones linked to its capacity to occupy ruminal space; b) the effective ruminal degradation rate for different feed fractions and c) those related to the disappearance probability from the rumen towards the lower tract (e.g. hydration capacity, specific weight, etc.).

Remarkable characteristics of this model are : 1) Takes into account the individual variability of animals in relation to the voluntary DM intake, 2) the degradation rates, although they are entered as a feed attribute, do not remain constant, changing according to the ruminal availability of nitrogen and carbohydrates, 3) the passage rates are modified according the actual rumen filling and 4) allows to predict the associative effects between different feeds.

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2 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

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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.

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ABSTRACT

The effects of the length of allowed grazing time (Experiment 1), length of starvation period before grazing (Experiment 2) on dry matter (DM) intake, ingestive behaviour and changes in DM rumen pool sizes during the first grazing bout were investigated in lactating Holstein-Friesian dairy cows. In Experiment 1 four lengths of allowed grazing time (1, 1.75, 2.50 and 3.25 h) after overnight starvation were compared. Increasing the length of the allowed grazing time significantly increased DM intake ($P<0.01$), the proportion of time spent actively eating ($P<0.01$) and DM rumen pool size after grazing ($P<0.05$). However, DM rumen pool size after grazing was smaller than that measured immediately before the start of grazing ($P<0.01$). Bite mass during the first hour of grazing was greater than in the following grazing time. Experiment 2 consisted of a factorial combination of two durations of starvation before grazing (16.5 and 2.5 h) and the presence or absence in the rumen of 12.5 kg of a synthetic indigestible material. DM intake as well as grazing time were greater after a starvation period of 16.5 h and were reduced by the presence in the rumen of indigestible material ($P<0.01$). The interaction between factors on grazing time, although not significant ($P<0.06$), supports the idea of a combination of signals controling meal size under grazing conditions. Bite mass was not significantly modified by period of starvation. DM rumen pool size after grazing was reduced by the placement in the rumen of synthetic indigestible material ($P<0.05$) and tended to be lower in cows with the larger period of starvation ($P<0.058$). In both experiments bite rate declined as the grazing session progressed, but bite rate was not affected by treatments.

INTRODUCTION

Daily dry matter (DM) intake of grazing ruminants can be expressed as the product of bite mass x biting rate x grazing time (Hodgson, 1985). Bite mass is a fundamental determinant of intake rate and it depends on sward structure (Arias *et al.*, 1990; Ungar *et al.*, 1991; Dougherty *et al.*, 1992; Laca *et al.*, 1992; 1994a; Flores *et al.*, 1993). Under grazing conditions, grazing is alternated by periods of fasting and with grazing lactating dairy cows, two main grazing bouts are normally observed, one in the morning and other in the evening. This pattern of grazing may be due to a short term fasting effect during milking (Rook *et al.*, 1994). Nevertheless, information on the effect of fasting and grazing time on ingestive behaviour is very limited (Demment *et al.*, 1995).

Voluntary DM intake of the first meal (Grovum, 1987; Gill and Romney, 1994; Forbes, 1995) as well as on a daily basis (Dulphy and Demarquilly, 1994; Van Soest, 1994) is believed to be controlled by metabolic (Mayne and Wright, 1988; Van Vuuren, 1993) and/or physical restrictions (Waldo, 1986). Under grazing conditions, ingestive behavioural restriction of the voluntary DM intake (Hodgson, 1985; Forbes, 1988; Demment *et al.*, 1995) may also play a role. It seems likely that a combination of signals rather than a single signal controls the initiation and

termination of a meal (Mbanya *et al.*, 1993; Forbes, 1995).

Two experiments were carried out to investigate the effect of length of the grazing period allowed after overnight starvation (Experiment 1), and the length of the starvation period and rumen fill before grazing (Experiment 2) on DM intake, ingestive behaviour and DM rumen pool size of lactating dairy cows grazing perennial ryegrass.

MATERIALS AND METHODS

The experiments were carried out in June (Experiment 1) and September (Experiment 2) 1995 at the experimental farm "De Ossekampen" of the Wageningen Agricultural University, The Netherlands.

Experimental design and treatments

In both experiments four cows were randomised across four treatments during four measurement periods according to a balanced 4x4 Latin square design (Steel and Torrie, 1980). The measurement periods had a duration of 28 h with intervening periods of 44 h. Treatments in Experiment 1 were the length of the grazing session: 1, 1.75, 2.50 and 3.25 h of grazing after overnight starvation. Experiment 2 consisted of a factorial combination of two durations of starvation before grazing (LS=16.5 and SS=2.5 h, respectively) and the presence (+) or absence (-) of 12.5 kg of a synthetic indigestible material in the rumen. The indigestible material in the rumen consisted of seventy-seven small nylon bags (0.15x0.08 m) filled with approximately 160 g of a non-degradable material (Shell polystyrene, particle size = 4x3 mm, specific density, 1.03 g ml^{-1}), and placed within a big bag (1.10x1.5 m) closed with a plastic tie. Hence, four treatments were compared: LS(+), LS(-), SS(+) and SS(-).A diagrammatic representation of the procedure followed during a measurement period in both experiments is shown in Figures 1 and 2 respectively. Note that in Experiment 1 the treatments were predetermined allowed grazing times, while in Experiment 2 the treatments were applied before grazing (Figure 2) and grazing time was one of the response variables measured.

Animal management.

Four lactating dairy cows previously prepared with a large rumen cannula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) were used. Mean live weights and daily milk yields over 4 consecutives milkings before and after each measurement period were 574 ± 29 and 618 ± 48, and 21.0 ± 3.5 and 18.3 ± 6.6 kg, for Experiments 1 and 2, respectively. The cows were milked twice each day (7.00 and 16.00 h) and ten days before the experiments started were trained to be led and graze whilst tethered. During the measurement periods the cows grazed individually, tethered within a circular plot of radius (r) = 6.0 m (Experiment 1) and r = 6.5 m (Experiment 2). During the intervening periods, the cows grazed freely in a contiguous pasture of perennial ryegrass which provided ample herbage. During the starvation periods cows had free access to water and mineral blocks.

Sward management

A pasture of predominantly perennial ryegrass (*Lolium perenne*), sown in 1987, was provided as the only feed during the experiments. Nitrogen fertilizer was applied on 7 April, 24 May, 27 July, 25 August (total: 150 kg N ha^{-1}) and manure on 8 April, 26 May and 26 July (total: approximately 20 m^3 manure ha^{-1}). Plots were mown on 22 May and 24 July and the duration of the regrowth at the start of the experiments was 4 weeks in Experiment 1 and 8 weeks in Experiment 2.

Grazing duration (hours)	Starvation 1	Grazing treatments		Starvation 2	
		Start		End	
1.00	18.00 *	* 09.30		10.30 *	18.15 *
1.75	18.00 *	* 09.00		10.45 *	18.30 *
2.50	18.00 *	* 08.30		11.00 *	18.45 *
3.25	18.00 *	* 08.00		11.15 *	19.00 *
Emptying *	1	2		3	4

Figure 1.
Diagramatic representation of the procedure followed during a measurement period : Experiment 1.

Treatments		Starvation 1		Grazing	Starvation 2	
Starvation (hours)	Bags			Start	End	
16.5	Yes	16.30 *		* 09.00	11.00 *	18.15 *
16.5	No	16.30 *		* 09.00	11.40 *	18.50 *
2.5	Yes		06.30	* 09.00	10.30 *	17.45 *
2.5	No		06.30	* 09.00	10.45 *	18.00 *
Emptying *		1		2	3	4

Figure 2.
Diagrammatic representation of the procedure followed during a measurement period : Experiment 2.

Sward measurements and herbage analysis

The sward was mown for sampling at a height of approximately 30 mm with a mower (Agria 3200; Agria-Werke, Möcknühl; cutting bar width: 0.65 m). Further references to herbage mass in the text are over 30 mm above ground level, except where indicated otherwise. Herbage mass before grazing was estimated in Experiment 1 by mowing two straight lines of 11.1 m length just outside of the grazing paddock and the residue after grazing by mowing four straight lines with an average length of 9.1 m. In Experiment 2, three straight lines of 12.0 m for herbage mass before grazing and four straight lines of 10.7 m on average for the residue were mown. After mowing, the forage was collected, placed in plastic bags, weighed and sampled for determination of DM content and chemical composition. DM intake was estimated as the difference between the herbage mass before grazing and the residue after grazing (DMI_F, Table 2 and 3) (Meijs, 1981). Sward height was measured using a plate metre (weight, 9.47 g; diameter, 0.1 m). Height determinations were made every 0.85 m parallel to the mown lines.
Forage samples were analysed for DM, ash, N and Neutral Detergent Fibre (NDF). DM was determined by drying the samples to constant weight in a oven at 65 °C, ash in an oven at 550 °C and N by the kjeldahl method with $CuSO_4$ as catalyst. NDF was measured according to the method of Goering and Van Soest (1970). DM content of the rumen samples was determined by freeze drying to constant weight.

Rumen content emptying and sampling

Manual emptying of the rumen contents was done 64 and 58 times in Experiment 1 and 2 respectively. The emptying sequence followed in each measurement period is presented in Figures 1 (Experiment 1) and 2 (Experiment 2). Solid rumen contents (solid) were removed by hand and placed in insulated containers that prevented the material cooling rapidly. Material not removable by hand (liquid) was collected with a plastic bottle and sieved (pore size, 0.04 mm^2) into a 40 l container. The liquid was weighed, sampled (approximately 1 l) and returned to the rumen, so that the cows' rumen remained completely empty for only two or three minutes. Solid material was weighed, mixed by hand in the insulated containers and two subsamples taken (approximately 400 g each); liquid was added to the solid subsamples in the same proportion (wet basis) as present in the rumen. During this sampling procedure the rumen cannula was kept closed to minimize rumen oxygenation and loss of heat. Samples were kept at -20 °C until analysis for DM content and chemical composition. DM clearance rate was estimated for each starvation period (Figures 1 and 2), assuming a first-order kinetics (Robinson *et al.*, 1986a), with one pool being cleared at a constant fractional rate. DM intake (DMI_R, Tables 2 and 3) was calculated as follows:

$$DMI_R = (RPAG - RPBG) + RPBG(1 - \exp^{(-k1\_GT)}) + CNGI$$

Where RPAG is estimated DM rumen pool after grazing (kg), RPBG is estimated DM rumen pool before grazing (kg), k1 is DM clearance rate (h^{-1}) during starvation 1 (Figures 1 and 2), GT is grazing time (h) and CNGI is clearance of the newly ingested grass (kg).

RPBG is the DM rumen pool measured in emptying 2 (RP2), corrected by clearance of DM

rumen content in the time elapsed between emptying and the start of grazing (Te1). RPAG is the DM rumen pool measured in emptying 3 (RP3) corrected by DM rumen content clearance rate in the time elapsed between end of grazing and emptying 3 (Te2). CNGI accounts for the rumen DM clearance through the grazing session of the grass that has just been ingested. To calculate CNGI the following assumptions were made: a uniform pattern of ingestion through the grazing session and a mean residence time of the particles ingested of 0.5 GT. The calculations were done as follows:

$$RPBG = RP2\_\exp^{(-k1\_Te1)};\ RPAG = RP\frac{3}{\exp^{(-k2\_Te2)}}$$

$$CNGI = [(RPAG - RPBG) + RPBG(1 - \exp^{(-k1\_GT)})] * (1 - \exp^{(-k2\_0.5GT)})$$

where: k2 is DM clearance rate (h^{-1}) during starvation 2 (Figures 1 and 2).

Grazing behaviour

Bite rate was recorded by an observer during the grazing sessions, over a period of 1 minute every ten minutes (Hodgson, 1982) for each cow individually. The sound when the forage was severed by the cows was chosen as the criterion to define and count bites because it was easily audible. In Experiment 1, the presence or absence of grazing activity by each cow was visually recorded at 2-minute intervals. When a cow was observed actively biting or searching with her head close to the sward, she was recorded as grazing. The effective grazing time (Table 2) was calculated by summation of each two-minute interval where grazing activity was positive. In Experiment 2 grazing time (Table 3) was the time elapsed between the cows being placed on the experimental plot and their voluntary cessation of grazing, when they were removed from the plot. Grazing was considered to have stopped voluntarily when the cows either lay down and started to ruminate, or when no further grazing activity was observed for 15 minutes continuously. Total bites was estimated as bite rate x grazing time, and bite rate during the effective grazing time (Experiment 1) as total bites divided by effective grazing time. Using the two estimates of DM intake obtained for each grazing session (DMI_F and DMI_R), herbage DM intake $bite^{-1}$ (BM_F and BM_R, respectively) were estimated from DMI divided by total bites. Similarly, two estimates of intake rate (IR_F and IR_R, kg 100 kg LW^{-1} h^{-1}) were calculated as a function of DM intake, effective grazing time (Experiment 1) or grazing time (Experiment 2) and individual mean live weight (LW).

Statistical analysis

Linear, quadratic and cubic effects of grazing time for each dependent variable were tested in Experiment 1, while the main effects and their interaction were estimated for Experiment 2. Changes of bite rate within treatments was analysed as repeated measurements over the same experimental unit. Regression analysis was performed considering the main effects as classes and each 10-minute time interval (one observation in each time interval) as the continuous variable. All the analyses were conducted using the GLM procedure of SAS (SAS Institute INC.,

1989).

RESULTS

Data describing the sward before and after grazing in both experiments are shown in Table 1. DM, organic matter (OM) and NDF content were lower in Experiment 1 than in Experiment 2, while the N content was higher in the second experiment (Table 1). The main response variables measured and estimated in Experiment 1 and Experiment 2 are shown in Tables 2 and 3 respectively. The estimations of DMI_F, BM_F and IR_F had a higher variability than those based on rumen measurements (DMI_R, BM_R, IR_R) as shown by the higher standard error of means (Tables 2 and 3). The estimations of DMI derived from rumen pool and sward measurements (DMI_F vs DMI_R, Tables 2 and 3) differed significantly ($P<0.01$) in Experiment 1, but not in Experiment 2.

Table 1. Mean sward and herbage values before and after aplications of treatments

	Experiment 1		Experiment 2	
Variable	Before	After	Before	After
Period	11-24 June		12-25 September	
DM hebage mass (>30 mm) (kg ha^{-1})	2673	2142	1785	1344
DM allowance (DMA) (kg $cows^{-1}$)	30,2	24,2	23,7	17,8
Sward height (mm)	214	157	97	71
Utilization of DMA (%)	19,9		24,6	
Chemical Composition (g kg DM^{-1})				
Dry matter (DM. G kg fresh wt^{-1})	246,9	258,3	256,5	289,2
Organic matter	921,5	922,8	941,6	942,6
Nitrogen	16,3	15,3	35,0	32,6
Neutral detergent fibre	501	508	551	568

DMA > 30 mm.

In Experiment 1 only linear effects of allowed grazing time were detected as significant. DMI_R increased and BM_R decreased linearly with the allowed grazing time in Experiment 1 (Table 2). Rumen DM pool size after grazing increased significantly as the allowed grazing time increased.

Table 2. DM intake from pasture (DMI_F) or rumen (DMI_R) measurements, DM intake rate (IR_F, IR_R), bite mass (BM_F, BM_R), bite rate, effective grazing time, total number of bites and DM rumen pool size in Experiment 1.

	Allowed grazing time (h).					
Variable	1	1,75	2,50	3,25	*s.e.*	*Significance*
DM intake (kg)						
DMI_F	4,75	5,76	4,57	8,90	2,46	NS
DMI_R	3,52	4,35	4,80	5,73	0,74	**
Intake rate (kg 100 kg LW^{-1} h^{-1})						
IR_F	0,81	0,57	0,31	0,47	0,22	*
IR_R	0,61	0,43	0,33	0,30	0,07	***
Bite mass (g $bite^{-1}$)						
BM_F	1,30	0,99	0,69	1,13	0,35	NS
BM_R	0,97	0,77	0,73	0,71	0,13	*
Effective grazing time (min)	60,0	103,2	120,0	149,0	15,0	***
Total number of bites	3670	5769	6726	7981	903	***
Bite rate (bites min^{-1})						
Average	61,2	53,5	45,1	41,3	3,40	***
In effective grazing time	61,2	56,0	56,2	53,0	2,57	***
in first hour	61,2	60,0	58,2	54,0	4,57	NS
DM rumen pools (kg)						
Emptying 1	12,0	11,3	11,6	11,4		
Emptying 2	4,83	4,64	4,66	4,99		
After grazing	7,86	8,23	8,32	9,17	0,80	*
Emptying 4	3,89	4,60	4,27	4,47	0,54	NS

*P < 0,05, **P < 0,01, ***P < 0,001; NS, not significant.

However, by the time emptying 4 was conducted 7.75 hours later, differences in rumen DM pool size were no longer significant (emptying 4, Table 1). The length of the allowed grazing session had a significant positive linear effect on the time spent actively eating (Table 2). Average bite rate, as well as bite rate during effective grazing time, declined with the length of the grazing session. Changes in bite rate during effective grazing time (Experiment 1) and bite rate (Experiment 2) throughout the grazing session were examined by regression of these variables determined at 10-minute intervals upon time since the start of grazing. No significant changes in bite rate were found during the first hour of grazing, but over the whole grazing session significant declines were observed: 0.41 and 0.51 bite $minute^{-1}$ for each successive 10-minute interval in Experiment 1 and 2, respectively.

Table 3. DM intake from pasture (DMI_F) or rumen (DMI_R) measurements, DM intake rate (IR_F, IR_R), bite mass (BM_F, BM_R), bite rate, effective grazing time, total number of bites and DM rumen pool size in Experiment 2.

	Starvation time (h)					Effect		
	LS	LS	SS	SS		Starvation	Indigestible residue in rumen	Interaction
Variable	(+)	(-)	(+)	(-)	s.e.	Significance		
DM intake (kg)								
DMI_F	4,95	7,80	4,20	6,43	1,72	NS	*	NS
DMI_R	5,36	7,83	3,41	5,48	0,83	**	***	NS
Intake rate (kg 100 kg LW^{-1} h^{-1})								
IR_F	0,41	0,48	0,48	0,57	0,12	NS	NS	NS
IR_R	0,43	0,47	0,35	0,49	0,04	NS	**	NS
Bite mass (g $bite^{-1}$)								
BM_F	0,71	0,80	0,84	0,97	0,17	NS	NS	NS
BM_R	0,75	0,82	0,63	0,84	0,09	NS	*	NS
Grazing time (min)	115,7	160,0	93,7	107,0	13,4	***	**	NS
Total number of bites	6872	9829	5459	6688	1367	*	*	NS
Bite rate (bites min^{-1})								
Average	59,5	61,0	59,7	61,5	5,29	NS	NS	NS
in first hour	63,0	62,2	62,0	63,3	4,23	NS	NS	NS
DM rumen pools (kg)								
Emptying 1	11,5	11,3						
Emptying 2	4,99	4,72	8,52	8,17	1,16	***	NS	NS
After grazing	9,20	11,14	10,83	12,75	1,27	NS	*	NS
Emptying 4	5,00	6,25	6,43	6,68	0,67	*	NS	NS

LS and SS. 16,5 and 2,5 h of starvation respectively, (+) and (-), presence or absence of bags within the rumen. For abbreviations see Table 2.

In Experiment 2, starvation as well as inclusion of bags affected DMI during the grazing session (DMI_R, Table 3). The length of the starvation period had no significant effect on BM_F and BM_R. Although IR_R followed the same trend as BM_R and DMI_R, the effect of indigestible material in the rumen on IR_R was more marked than starvation itself. Rumen DM pool size before grazing (emptying 2, Table 3) was significantly different between treatments (4.85 vs 8.34, $P<0.001$, LS vs SS). Rumen DM pool size after grazing tended to be lower in cows starved longer and was significantly reduced by the placement of bags in the rumen (Table 3). Cows on LS treatments spent more time grazing, while the placement of bags within the rumen significantly reduced voluntary grazing time. However the effect of indigestible material in the rumen tended to be different ($P<0.06$), following different periods of starvation. Neither bite rate in the first hour of grazing nor average bite rate were significantly different between treatments (Table 3).

DISCUSSION

Sward measurements

To avoid a possible reduction in DM intake, during each grazing session individual cows were allocated to areas of sufficient size so that the utilization of DM offered was less than 50 % (Hodgson, 1990). However, despite there being no detectable differences in the height of stubbles

remaining after mowing, and an absence of any height measurement on the grazed area below 30 mm, the large variability in determinations of DM mass before and after grazing and the low percentage utilization of herbage offered (Meijs, 1981), resulted in estimates of DM intake differing markedly from those obtained from rumen pool measurements.. Because of what we considered to be poor reliability of the estimates of intake derived from sward measurements, discussion of DM intake, BM and IR in both experiments will be confined to those derived from rumen pool measurements.

DM intake, intake rate and DM rumen pools

In Experiment 1, grazing behaviour changed when allowed grazing time was increased. In the first hour of grazing, cows took large bites (>0.9 g bite^{-1}) at a fast rate (> 61 bites minute^{-1}) resulting in a high rate of intake (>0.6 kg 100 kg LW^{-1} h^{-1}). As grazing time was extended to 1.75 hour, bite mass declined markedly (-0.2 g bite^{-1}), although further extension of grazing time to 2.50 or 3.25 h resulted in little further decline. The relatively small increments in DM consumed as available grazing time was extended beyond 1 hour was mainly the result of cows failing to increase their grazing time to the maximum possible. Although rumen DM pool size after grazing increased linearly with the allowed grazing time it remained significantly lower than DM rumen pool sizes recorded the previous afternoon (P<0.01, emptying 1 vs rumen DM pool size after grazing; Table 2). The values observed in emptying 1 (average 11.59 kg DM, approximately 20 g DM kg LW^{-1}) were similar to the maximum values reported by Bosch (1991) when offering silage *ad libitum* and at the higher level of intake reported by Robinson *et al.* (1987), but were lower than the values reported by Harnell and Satter (1979), all measured in lactating dairy cows. However rumen DM pool size after grazing (Table 2), was far below the maximum potential rumen capacity, indicating that the cows in this experiment stopped grazing or interrupted the first meal before their ruminal capacity was reached (Gill *et al.*, 1988; Thiago *et al.*, 1992; Van Vuuren, 1993).

In Experiment 2 the longer period of starvation increased DM intake while the presence of bags in the rumen decreased DM intake (Table 3). On average cows on treatment LS showed an increment in DM intake of around 48%, mainly due to the longer grazing time exhibited and to a lesser extent due to a non significant increase in BM_R (Table 3). There was no interaction effect on DM intake. However, there was a suggestion of an effect (P<0.06) on IR_R. Whilst, the effect of starvation time on DM intake in cows without bags in the rumen (LS(-) vs SS(-)) can be almost entirely explained by differences in grazing time, in cows with bags in (LS(+) vs SS(+)) the variation on DM intake can be attributed to changes in both grazing time and in bite mass, in approximately the same proportion (Table 3). Regarding rumen DM pool size after grazing, a significant effect of indigestible material in the rumen and a more moderate effect of duration of starvation was observed. The rumen DM pool size after grazing in LS animals tended to be lower than in SS animals (10.2 vs 11.8 kg, respectively; P<0.058). It should be noted that receptors in the rumen wall can respond to stretch or mechanical stimulation (Gill *et al.*, 1988) and polystyrene material, in addition to their fill effect, may have stimulated the tactile receptors of the rumen wall and thus initiated rumination behaviour (Baumont *et al.*, 1990). Otherwise, the smaller rumen DM pool size before grazing (Table 3, emptying 2) associated with a higher absolute DMI_R of LS animals, may have led to different rumen fermentation patterns (Robinson *et al.*, 1986b), signalling the end of the meal at a lower DM rumen pool size than in SS animals.

Moreover, the threshold level tolerated by the cows could have been different for both starvation times (Grovum, 1987; Faverdin *et al.,* 1995). These different effects may have been confounded in this experiment. Estimated IR_R in both experiments were slightly lower than the 0.5 kg DM 100 kg LW^{-1} h^{-1} proposed by Dougherty *et al.* (1992) as potential intake rate, after hunger is alleviated.

Grazing time

The increase in effective grazing time was not proportional to the increase in allowed grazing time (Table 2). In Experiment 2, starvation itself had an effect on grazing time but the magnitude of the effect tended to vary depending on whether or not bags were present in the rumen (Table 3). The effect of indigestible material in the rumen was dependent on the starvation time: in LS cows the inclusion of indigestible material led to a reduction in grazing time of 44.3 minutes, while in SS cows this reduction was only 13.4 minutes. Greenwood and Demment (1988) found similar trends when comparing the effect of two times of fasting (36 vs 1 hour) on grazing behaviour of steers with most of the difference attributable to a much longer initial grazing bout. The tendency for an interaction between duration of starvation and indigestible material in the rumen (Table 3) suggests the possibility of additive effects of treatments on grazing time (Mbanya *et al.,* 1993; Gill and Romney, 1994) although other factors may have been involved (see discussion above).

Bite mass

Bite mass has been described as the main component of grazing behaviour determining intake rate (Hodgson, 1985; Forbes, 1988; Laca *et al.,* 1992; 1994a). In Experiment 1, bite mass declined linearly throughout the grazing session. Dougherty *et al.* (1987) found the same tendency for Angus heifers grazing tall fescue, but not lucerne swards. In Experiment 2 there was no effect of length of starvation on bite mass, which is in agreement with the results of Greenwood and Demment (1988) and supports the hypothesis that bite mass is linked to sward characteristics (Arias *et al.,* 1990; Ungar *et al.,* 1991; Dougherty *et al.,* 1992; Laca *et al.,* 1992; 1994a; Flores *et al.,* 1993). However, the inclusion of bags in the rumen did significantly reduce BM_R (Table 3). Although field studies of grazing ruminants have generally found an increase in bite rate with decreasing bite mass (Hodgson, 1985; Forbes 1988), data from this experiment did not indicate a close relationship between these two variables. Although Laca *et al.* (1994b) observed that patch depletion affected bite mass but not bite rate, they are not independent, the most obvious functional link being the fact that larger bites would require more ingestive mastication. Nevertheless this relationship may not be linear, since Laca *et al.* (1994a) observed that the cattle were able to chew and bite with the same jaw movement. This indicates the existence of a critical bite mass below which, time per bite and total number of chewing movements does not change. In the experiments that supported that model, critical bite mass was found to be around 2 mg DM kg LW^{-1}, which is greater than the values estimated in this experiment (1.38 and 1.23 mg DM kg LW^{-1} for Experiment 1 and Experiment 2, respectively). Extrapolation from this work has to be done carefully, since these authors worked at feeding station level (Laca *et al.,* 1994a), but the conceptual model could be useful for understanding the apparent independence of bite size and biting rate that we observed in our experiments.

Bite rate

The average bite rate declined throughout the longer grazing sessions, though when corrected for effective grazing time, the changes were less. Analysis of the bite rate changes within the first hour of grazing showed slopes not significantly different from 0, in both experiments. A decline in bite rate as grazing session progressed has been observed previously in grazing experiments (Chacon *et al.*, 1976; Jamieson and Hodgson , 1979; Dougherty *et al.*, 1987; 1989b) but not invariably (Forbes and Hodgson, 1985). The absence of an effect of duration of starvation on bite rate in Experiment 2 (Table 3) is in contrast with the results of Dougherty *et al.* (1989a) and Greenwood and Demment (1988). However, it is also possible that 2.5 hours of starvation and the emptying procedure itself induced enough "hunger feeling" for the cows to start grazing in the same way as those with larger starvation times (Dougherty *et al.*, 1989a).

CONCLUSION

Allowed grazing time had a linear effect on the proportion of the available time spent actively eating and on the DM rumen pool size after grazing. However the observed DM rumen pools after grazing do not support the idea of DM rumen fill as the main factor signalling the end of the first meal in this experiment. Treatments had no effect on average bite rate, although bite rate was found to decline as the grazing session progressed. Different lengths of starvation before grazing did not affect the average bite mass during the subsequent grazing bout, while the placement of inert material within the rumen did reduce bite mass. Length of period of starvation and placement of inert material in the rumen both had a significant effect on the duration of the first grazing bout. A slight interactive effect ($P<0.06$) of the duration of starvation and indigestible material in the rumen on the duration of first grazing bout supports the concept of a combination of factors signalling the termination of a meal.

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3

EFFECT OF ALLOWED GRAZING TIME, INERT RUMEN BULK AND LENGTH OF STARVATION BEFORE GRAZING, ON THE WEIGHT, COMPOSITION AND FERMENTATIVE END-PRODUCTS OF THE RUMEN CONTENTS OF LACTATING DAIRY COWS

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ABSTRACT

The effect of the length of the allowed grazing time (Experiment 1) and length of starvation time and placement in the rumen of inert bulk material before grazing (Experiment 2), on liquid and particulate rumen pool sizes, composition and fermentability was investigated. In Experiment 1 four lengths of allowed grazing time (1, 1.75, 2.50 and 3.25 h) after overnight starvation were compared. The allowed grazing time did not have any significant effect on total and liquid rumen pool sizes after grazing but did have ($P<0.05$) on DM and OM (slope, 0.5 kg h-1) rumen pool sizes. The non-significant differences between the volatile fatty acids (VFA) rumen pool sizes before and after 1 hour of grazing indicate a delay in the availability of the more rapidly fermentable substrate for the microorganisms. The total VFA rumen pool sizes increased significantly ($P<0.01$; slope, 1.88 mol h-1) with the allowed grazing time, which suggest that these fermentation products may be involved in the control of the grazing time in later stages during the day. Experiment 2 consisted of a factorial combination of two length of starvation before grazing (16.5 and 2.5 h) and the presence or absence in the rumen of 12.5 kg of a synthetic indigestible material. The duration of starvation before grazing did not affect significantly the particulate, ammonia and VFA rumen pool sizes after grazing except for propionic acid which was reduced ($P<0.05$) by the larger starvation time. The inclusion of inert bulk material in the rumen before grazing significantly reduced ($P<0.05$) the total, liquid, DM, OM and ammonia rumen pool sizes but not the VFA rumen pool sizes after grazing. High levels of ammonia as well as total rumen contents may be involved in the control of the grazing time in this experiment.

INTRODUCTION

The analysis of the mechanisms that control voluntary dry matter (DM) intake in ruminants has received special attention (example: Conrad et al., 1964; Balch and Campling, 1966; Grovum 1987; Gill et al, 1988, Dulphy and Demarquilly, 1994; Forbes, 1995), since feed intake is the predominant factor determining animal performance (Illius and Jessop, 1996). The theories of physical regulation of DM intake have been supported both by the presence of stretch and mechanical receptors in the ruminal wall (Baumont et al., 1990; Forbes 1995), and by the observed depression on DM intake when the rumen capacity has been artificially reduced (Faverdin et. al., 1995; Dado and Allen, 1995). Alternatively to this theory, the concentration of fermentation products has also been proposed as playing a role in the control of voluntary DM intake of silages (Gill et al, 1988; Van Os, 1997), fresh forages (Van Vuuren, 1993), and roughage in general (Grovum, 1987; Illius and Jessop, 1996), but it seems that a combination of signals emanated from various types of receptors and integrated at the

nervous central system (Mbanya et al., 1993; Forbes, 1996) rather than single signals operating isolated from one another, are responsible for the ultimate control of voluntary DM intake. Under grazing conditions, despite the intensity of research (Hodgson, 1985; Forbes, 1988; Laca et al., 1994) and modelling (Illius and Gordon 1991; Parsons et al., 1994; Demment et al., 1995; Newman et al., 1995) of control of DM intake, little progress has been made to determine the factors that control grazing time.

It has been suggested that intake may be limited by the capacity of reticulum-rumen to clear feed by degradation and/or passage (Tamminga and Van Vuuren, 1996), and a slow rate of particle breakdown might hamper the clearance of material from the rumen. Rate of particle break-down does not seem to be an important factor since particle size reduction during eating often causes over 50 % of the particles to become small enough to leave the rumen (Ullyat et al., 1986). However, the work reported by Waghorn et al (1986; 1989) and recently by Kusmartono et al. (1997) suggest that the chewing efficiency during eating of cows and deer fed fresh ryegrass was below 50 %. Unfortunately, there is still a lack of quantitative information on ingestive mastication during grazing (Laca et al., 1994) and its consequences for digestion and passage.
In the current paper we examine the effect of the length of the allowed grazing session, duration of starvation time before grazing and the placement of inert material within the rumen, on the liquid and particulate rumen pool sizes, composition and fermentability. The effect of these treatments on grazing ingestive behaviour and dry matter intake has been reported elsewhere (Chilibroste et al., 1997).

MATERIAL AND METHODS

Treatments and design

Two experiments were carried out in June (Experiment 1) and September (Experiment 2) 1995 at the experimental farm "De Ossekampen" of the Wageningen Agricultural University, The Netherlands. In both experiments, four cows were randomised across four treatments during four measurement periods according to a balanced 4*4 Latin square design (Steel and Torrie, 1980). Treatments in Experiment 1 concerned the length of the grazing session: 1, 1.75, 2.50 and 3.25 h of grazing, after overnight starvation. The linear effects of grazing time on rumen pool sizes, composition and fermentability were tested. Experiment 2 consisted of a factorial combination of two durations of starvation before grazing (SS=2.5 and LS=16.5 h, respectively) and the presence (+) or absence (-) of 12.5 kg of a synthetic indigestible material in the rumen. Hence, four treatments were compared: SS(+), SS(-), LS(+) and LS(-). The effect of the starvation time, addition of inert rumen bulk and their interaction on rumen pool sizes, composition and fermentability were tested. All the statistical analyses were performed using the GLM procedure of SAS (SAS Institute INC., 1989).

Animals and experimental periods

Four lactating dairy cows previously prepared with a large rumen cannula (10 cm id., Bar Diamond Inc., Parma, Idaho, USA) were used. Mean live weights and daily milk yields over 4

consecutive milkings before and after each measurement period were 574 • 29 and 618 • 48, and 21.0 • 3.5 and 18.3 • 6.6 kg, for Experiments 1 and 2, respectively. The process of evacuation, weighing, sampling and returning of whole rumen contents to the cows, will be referred to in this paper as emptying. The sequence of emptying is numbered from 1 to 4: emptying 1 (EMT1) was done before the first starvation period, emptying 2 (EMT2) the following morning after starvation and before grazing, emptying 3 (EMT3) immediately after the experimental grazing session, and emptying 4 (EMT4) after the period of starvation that followed EMT3. A detailed description of animal and sward management, the rumen emptying procedure and the statistical analysis carried out in both experiments has been published elsewhere (Chilibroste et al., 1997).

Sampling and analysis

As described by Robinson et al. (1987) all the digesta that could be removed by hand (MAT) was placed in an insulated container and the remaining material (BAILABLE) collected with a plastic bottle. Although this procedure allowed the separation of MAT and BAILABLE fractions to a certain extent, we always observed a quick drainage of liquid to the bottom of the container, that made the sampling procedure more difficult and inaccurate. Hence, a slight modification in the procedure was introduced during Experiment 2, by placing a double lace curtain to cover the bottom and the sides of the container. The drained liquid was poured through a sieve with a mesh size of 0.04 mm2, into the bucket with the BAILABLE fraction. Samples of the MAT fraction (approximately 2 kg) were collected and kept frozen at –20°C for wet sieve analysis.

Immediately before each rumen emptying, samples of rumen fluid (approximately 250 ml) were collected with a 85 cm plastic tube (2.5 cm diameter) closed at the bottom and with about 270 holes (1.5 mm diameter) drilled in the bottom 27 cm. This tube was inserted into the rumen through the rumen cannula and positioned in such a way that the bottom of the tube reached the liquid phase in the ventral rumen sac. Rumen fluid was removed by a flexible tube (0.5 cm diameter) which was placed into the plastic tube and was connected to a plastic bottle. The collected rumen fluid was sieved through a sieve with apertures of 0.04 mm2, and samples were taken for pH, ammonia and volatile fatty acids (VFA). pH was measured immediately after collection (pH electrode type 62, Testo 252, Testo GmbH & Co, Germany). A portion of the sample (5 ml) was mixed with 5 ml of trichloroacetic acid and kept frozen at –20°C until analysis for ammonia. For analysis, the samples were thawed at room temperature, centrifuged for 10 minutes at 4200 rpm (Sigma 2-15, Laborzentrifugen GmbH, Germany), and the ammonia concentration in the supernatant determined by a spectrophotometer at an wavelength of 623 nm (Beckmann DU 64, Soft Pac module Quant II, Beckmann Instruments, Inc, USA). Another portion (10 ml) of the rumen liquor sample was acidified with 0.5 ml 85% phosphoric acid and kept frozen at -20• C pending analysis for VFA. For VFA analysis the samples were thawed at room temperature and centrifuged for 10 minutes at 12000 rpm (IEC Centra-M, International Equipment Company, USA). Subsequently 500 • l of the supernatant was mixed with 200 • l water and 300 • l of an internal standard (iso-caproic acid). One • l of this mixture was injected into a gas-liquid chromatography (Packard Becker model 419, packed column filled with chromosorb 101, carrier gas N2 saturated with formic acid, temperature 190 • C). The analysed VFA were: acetic acid (C2), propionic (C3), iso-butyric (iC4), butyric (C4), iso-valeric (iC5) and valeric acid (C5). The total concentration of VFA in the rumen liquor was calculated as the sum of

C2, C3, C4, iC4 and iC5. The relationship between non-glucogenic and glucogenic VFA was calculated as the ratio between C2+C4 and C3 (NGR).
Rumen samples were analysed for DM, ash, N, Neutral Detergent Fibre (NDF) and Acid Detergent Lignin (ADL). DM content of the rumen samples was determined by freeze drying to constant weight, ash in an oven at 550 oC and N by the Kjeldahl method with CuSO4 as catalyst. NDF and ADL were determined according to the method of Goering and Van Soest (1970).

Sieve analysis

Fresh rumen samples (MAT fraction) collected after grazing in Experiment 1 were wet sieved (Retsch AS-200 Control, Retsch GmbH & Co. KG, Haan, Germany). Three sieves were used with sieve apertures of 5.0, 2.5 and 1.25 mm. The samples were sieved for 15 minutes with an amplitude of 2 mm and a water flow of about 10 l min-1. The wet sieve procedure was as described by Bosch et al. (1992) with 3 repetitions of 5 minutes each.

RESULTS

Experiment 1

The changes in total, DM, OM, NDF and liquid rumen pool size through the sequence of rumen emptyings are shown in Table 1. The overnight starvation resulted in a decrease in the

Table 1. Experiment 1. Total, liquid, dry-matter (DM), organic matter (OM) and neutral detergent fiber (NDF) rumen pools, fraction removable by hand (MAT, g.kg^{-1} DM) and DM rumen content (DMC g.kg^{-1}). Least square means.

			EMT_3							
Variable	**EMT_1**	**EMT_2**	**G 1**	**G 1,75**	**G 2,5**	**G 3,25**	**s.e.m.**	**Slope**	**P**	**EMT_4**
Rumen pool (kg)										
Total	94,1	50,6	74,0	77,0	75,1	79,7	7,57	2,0	NS	53,6
Liquid	83,4	45,9	65,3	66,7	63,2	65,0	5,84	-0,6	NS	49,3
DM	11,6	4,8	7,9	8,2	8,3	9,2	0,80	0,5	*	4,3
OM	10,3	4,2	7,0	7,3	7,4	8,1	0,65	0,5	*	3,8
NDF	5,8	2,5	3,7	4,0	4,0	4,4	0,35	0,3	NS	2,2
Rumen characteristics										
DMC (g. Kg^{-1})	114	95	108	107	110	115	6,8	3,0	NS	80
MAT (g. Kg^{-1} DM)	970	926	927	935	946	942	18,2	7,0	NS	845

EMT1 to -EMT2 state for emptyings 1,2,3 and 4 respectively; G 1, G 1,75, G 2,5 and G 3,25 state for grazing time: 1, 1,75, 2,5 and 3,25 h respectively; Slope is change in rumen pool or rumen characteristics per hour of grazing time (kg h^{-1}, g kg^{-1} h^{-1} or g kg^{-1} DM h^{-1}).
*P<0,05; NS, not significant.

size as well as the proportions between the various rumen fractions. The total rumen pool size was reduced because the continuous clearance of material from the rumen (EMT1 to EMT2, Table 1), and the rumen DM content and the proportion of the total rumen pool present as a MAT had also significantly diminished. The DM, OM and NDF rumen pools decreased between EMT1 and EMT2, but not at the same rate, due to their different susceptibility to

clearance from the rumen. It is noteworthy that the rumen pool sizes after grazing were significantly smaller than the observed values at the beginning of the starvation period (EMT3 vs EMT1, Table 1). Otherwise, while the total and liquid rumen pool sizes were not significantly different between grazing treatments and did not show a significant linear trend

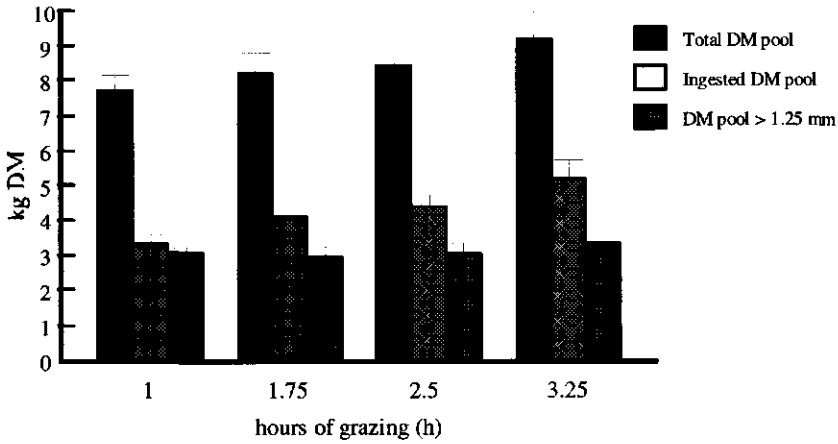

Figure 1. Experiment 1. Dry-Matter (DM) rumen pools after grazing (kg DM): total, new material ingested during the grazing session and rumen pool larger than 1.25 mm.

with the allowed grazing time, the DM and OM rumen pool sizes increased significantly.

In Figure 1 the total DM rumen pool after grazing, the DM rumen pool just ingested during the grazing session and the DM rumen pool > 1.25 mm are shown. The newly ingested DM pool size was estimated as the difference between the total rumen DM pool after grazing minus the DM pool before grazing corrected for the clearance of material during the grazing session (Chilibroste et al., 1997). The DM pool of particles > 1.25 mm (average, 3.01 kg) was not significantly different between treatments. The proportion of the newly ingested pool represented by particles > 1.25 mm declined significantly ($P<0.01$) with the allowed grazing time. The rumen particle size distribution is presented in Figure 2. It is remarkable that a relatively large proportion of the particles > 1.25 mm was retained on a sieve aperture of 5 mm, with no significant differences between grazing treatments. However, the proportion of particles retained between 5 and 1.25 mm declined linearly ($P<0.01$) with the allowed grazing time.

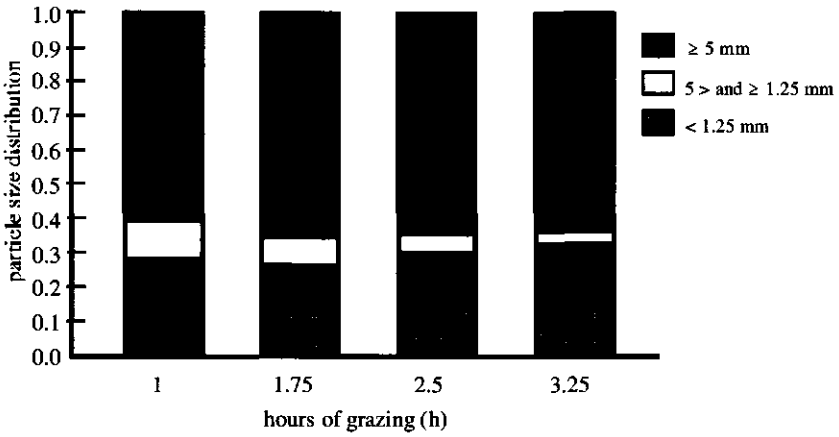

Figure 2. Experiment 1. Rumen particle size distribution after grazing.

The changes in pool sizes of rumen fermentation products at the different emptyings, are shown in Table 2. The effect of the allowed grazing periods on these pools are also shown in Table 2.

The rumen pool sizes increased linearly with the allowed grazing time, while pH and NGR in the ruminal liquor declined. It must be noted that unlike the observations for the particulate and liquid rumen pools (Table 1), the total, C2, C3, C4 and minor VFA rumen pool sizes after 3.25 hours of grazing, were not significantly different from the values measured initially (EMT1, Table 2). The non-significant difference between the pool sizes before and after 1 hour of grazing (EMT2 vs EMT3 1 h, Table 2) is also remarkable.

Experiment 2

The changes in the particulate and liquid rumen pools at the different emptying are shown in

Table 2. Experiment 1. pH, pool sizes of ammonia (NH_3P,g), acetic acid (C_2P, mol), propionic acid (C_3P, mol), butyric acid (C_4P, mol), isobutyric acid (C_4P, mol), isovaleric acid (iC_5P, mol) and total volatile fatty acids (TVFAP, mol) and the ratio of non-glucogenic to glucogenic (($C_2P + C_4P$)/C_3P; NGR) fatty acids in emptyings one (EMT_1), two (EMT_2), three (EMT_3) and four (EMT_4). Least square means.

			EMT3							
Variable	**EMT_1**	**EMT_2**	**G 1**	**G 1,75**	**G 2,5**	**G 3,25**	**s.e.m.**	**Slope**	**P**	**EMT_4**
pH	6,14	7,05	6,57	6,43	6,31	6,04	0,17	-0,23	**	6,97
NH_3P	7,78	2,79	3,85	6,20	5,61	7,18	1,36	1,30	*	1,58
C_2P	4,77	1,75	2,33	3,28	3,72	4,89	0,34	1,08	**	1,65
C_3P	1,71	0,42	0,69	1,15	1,35	1,78	0,11	0,46	**	0,42
C_4P	1,07	0,18	0,28	0,51	0,77	1,00	0,06	0,31	**	0,23
iC_4P	0,04	0,02	0,02	0,03	0,03	0,05	0,01	0,01	*	0,02
C_5P	0,08	0,01	0,03	0,02	0,04	0,07	0,02	0,02	*	0,01
iC_5P	0,10	0,04	0,04	0,06	0,06	0,07	0,02	0,01	*	0,03
TVFAP	7,69	2,42	3,37	5,04	5,84	7,81	0,50	1,88	**	2,35
NGR	3,41	4,62	3,80	3,30	3,28	3,29	0,24	-0,20	*	4,46

For abbreviations see Table 1.
*P<0,05; **P<0,01

Table 3. Although the length of starvation before grazing significantly reduced the rumen pool sizes at the time of EMT2, the differences were no longer significant after the grazing session ($P< 0.01$). Otherwise, the inclusion of inert material within the rumen before grazing had a significant effect on total, liquid and OM rumen pool after grazing. Unlike Experiment 1, the rumen pool sizes after grazing were not significantly different from the pools priors to the large starvation period.

The pool sizes of the rumen fermentation products at the different emptyings, are shown in Table 4. The duration of starvation before grazing significantly affected the total amount of fermentation products at the time of EMT2. This effect remained after grazing for pH, C3 pool size and NGR ratio, but had only a slight effect ($P<0.10$) on C2, C4 and total VFA rumen pool sizes. Otherwise, the inclusion of inert material within the rumen, significantly reduced the ammonia rumen pools size after grazing (Table 4).

DISCUSSION

Procedure

The rumen of each cow was emptied with a particularly high frequency: 4 emptyings per measurement period of 28 hours. We did not critically test whether such a frequency of rumen emptying could have had a negative effect on rumen fermentation patterns, animal ingestive behaviour or performance. However, no problems were detected with any of the cows. Since the ruminal system is highly buffered along with having a large reducing capacity, it seems to have been able to maintain anaerobiosis during emptying without serious consequences to ruminal micro-organisms or their fermentation patterns (Towne et al., 1986; Moloney et al., 1993). Otherwise, the modification introduced in the rumen emptying procedure in Experiment 2, aimed to improve the separation of the MAT and BAILABLE fractions, reduced the time required for hand mixing. The sampling was more accurate as indicated by the absolute average difference (%) in rumen DM content between duplicates (2.9 vs 9.1 %, for Experiment 2 and 1, respectively).

Table 3. Experiment 2. Total, liquid, dry-matter (DM), organic matter (OM) and neutral detergent fiber (NDF) rumen pools, fraction removable by hand (MAT, $g.kg^{-1}$ DM) and DM rumen content (DMC $g.kg^{-1}$). Least square means.

		EMT_2			EMT_3				Significance				
Variable	**EMT_1**	**LS**	**SS**	**Starv**	**LS(+)**	**LS(-)**	**SS(+)**	**SS(-)**	**s.e.m.**	**Starv**	**Bag**	**Int.**	**EMT_4**
Rumen pool (kg)													
Total	96,5	55,6	72,6	***	81,5	103,3	90,3	98,7	10,9	NS	*	NS	63,3
Liquid	85,1	50,8	64,3	***	72,5	93,0	79,8	87,0	9,72	NS	*	**	57,2
DM	11,4	4,8	8,3	***	9,2	11,1	10,8	12,7	1,27	NS	*	NS	6,1
OM	11,1	4,4	7,9	***	8,8	10,8	10,5	12,2	1,29	NS	*	NS	5,6
NDF	5,2	2,3	3,8	***	4,6	5,6	5,4	5,8	0,35	NS	NS	NS	3,3
Rumen characteristics													
DMC ($g.Kg^{-1}$)	900	758	907	***	865	808	885	808	317	NS	*	NS	779
MAT ($g.Kg^{-1}$ DM)	118	87	114	***	108	100	115	117	63	**	NS	NS	95

SS, LS; 2,5 and 16,6 h of starvation respectively; (+), (-): presence or absence of Bag within the rumen respectively;
Starv, Bag, Int: starvation, bag and interaction effect, respectively. For other abbreviations see Table 1.
*P<0,05; **P<0,01; ***P<0,001; NS, not significant.

Table 4. Experiment 2. pH, pool sizes of ammonia (NH_3P,g), acetic acid (C_2P, mol), propionic acid (C_3P, mol), butyric acid (C_4P, mol), isobutyric acid (C_4P, mol), isovaleric acid (iC_5P, mol) and total volatile fatty acids (TVFAP, mol) and the ratio of non-glucogenic to glucogenic (($C_2P + C_4P$)/C_3P; NGR) fatty acids in emptyings one (EMT_1), two (EMT_2), three (EMT_3) and four (EMT_4). Least square means.

		EMT_2			EMT_3				Significance				
Variable	**EMT_1**	**LS**	**SS**	**Starv**	**LS(+)**	**LS(-)**	**SS(+)**	**SS(-)**	**s.e.m.**	**Starv**	**Bag**	**Int.**	**EMT_4**
pH	6,2	7,3	6,8	**	7,0	6,6	6,7	6,6	0,12	**	*	NS	6,8
NH_3P	40,90	8,54	17,71	*	22,97	39,07	27,31	34,04	5,73	NS	*	NS	11,64
C_2P	6,13	1,40	4,02	***	2,87	4,17	4,75	4,80	1,06	NS	NS	NS	3,07
C_3P	1,81	0,30	1,08	***	0,63	0,93	1,29	1,27	0,35	*	NS	NS	0,83
C_4P	1,12	0,18	0,67	***	0,33	0,58	0,78	0,72	0,24	NS	NS	NS	0,53
iC_4P	0,11	0,03	0,07	*	0,05	0,07	0,1	0,09	0,04	NS	NS	NS	0,07
C_5P	0,16	0,02	0,09	***	0,07	0,09	0,11	0,10	0,04	NS	NS	NS	ne
iC_5P	0,23	0,06	0,12	*	0,08	0,11	0,15	0,13	0,05	NS	NS	NS	0,10
TVFAP	9,41	1,97	5,96	***	3,97	5,87	7,07	7,01	1,75	NS	NS	NS	4,60
NGR	4,22	5,19	4,43	**	5,2	5,1	4,3	4,4	0,36	*	NS	NS	4,40

ne, non-estimable; for other abbreviations see Tables 2 and 3.
*P<0,05; **P<0,01; ***P<0,001; NS, not significant.

Rumen pool sizes and characteristics

The proportion of the total rumen content present as MAT decreased in both experiments along with the size of the total rumen content. The faster decline of DM compared to non-DM rumen content, can be seen as a physiological adaptation of the rumen capacity either to changes in total dry matter intake (Hartnell and Satter, 1979; Robinson et al., 1987) or deprivation of food for several hours (this experiment). A higher proportion of a floating MAT fraction as the rumen become full, should have consequences for the trapping of small particles (Allen, 1996), preventing high values of passage rate at high levels of intake (Robinson et al., 1987).

In both experiments, the measured total rumen content before the first starvation period (approximately 160 g of rumen content per kg live weight), were comparable with the values reported for dairy cows fed grass silages (Gasa et al., 1991; Bosch et al., 1992) and alfalfa hay (Hartnell and Satter, 1979; Robinson et al., 1987) as basal diets, and somewhat higher than the values reported by De Visser et al., (1992; 1993), Johnson and Combs (1991, 1992) and Shaver et al., (1986; 1988) also for dairy cows. However, DM and OM rumen pools were somewhat lower (for the comparable total rumen content weighs), than the values reported by the previously mentioned authors. The apparent discrepancy between both comparisons (wet and dry basis) is based on the lower rumen DM content of our cows, since most of these authors worked with winter rations (40-60 % DM) with a medium to high level of supplementation. This high level of supplementation has been suggested to be responsible for higher rumen DM content (Bosch, 1991; Gasa et al., 1991).

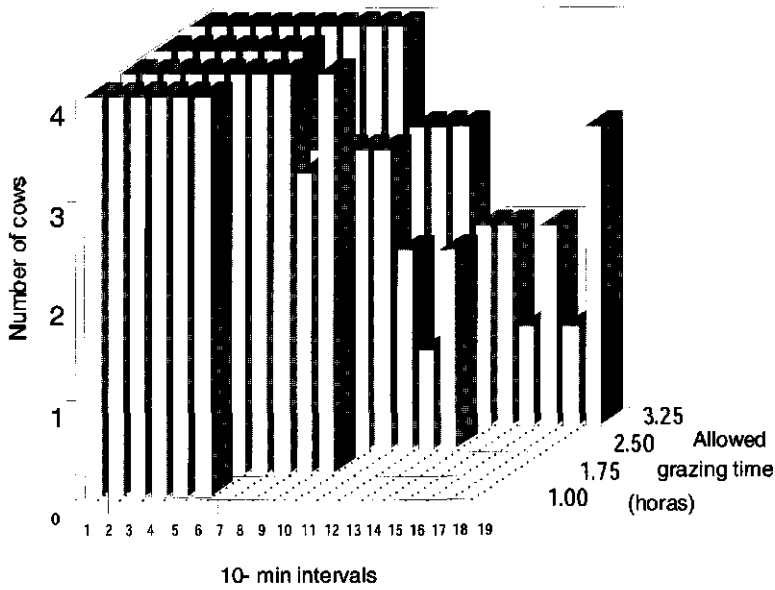

Figure 3 Number of cows grazing during the allowed grazing time. Experiment I.

The different trends observed for the wet and dry rumen pool sizes after grazing (Table 1) could be ascribed to the non-significant (P=0.12) increase in rumen DM content for the allowed grazing period. This increase might be related to the grazing patterns exhibited by the cows (Figure 3), which seemed to require at least one period of rumination after approximately one and a half hours after the start of the grazing session. Although we did not systematically record rumination activity (Chilibroste et al., 1997), we normally observed that the cows lay down and started rumination, when not actively biting. The rumen DM content after grazing of LS cows in Experiment 2, was also around 10%, while SS cows showed higher values (Table 3). Relatively low values of rumen DM content before (8.4 % DM), during and immediately after eating (10.6 % DM) fresh ryegrass have been reported by Waghorn et al. (1986) and Waghorn et al. (1989). The fact that the cows may have required a period of rumination to re-chew the ingested material indicates that the ingestive chewing was not as efficient as expected (Ulyatt et al., 1986; Waghorn et al.,1986). It has been shown that cattle are able to reduce the amount of chewing during eating to increase intake rate (Parsons et al., 1994), particularly after a period of fasting (Greenwood and Demment, 1988). If we assume that the proportion of rumen particles over 1.25 mm after 16.5 hours of starvation was about 15 % (Bosch and Bruining 1995), the wet sieve analysis revealed that about 75 % of the ingested new material during the grazing session was larger than 1.25 mm, after 1 hour of grazing (Figure 1). As the grazing session continued and a period of rumination was observed (Figure 3), this figure declined to approximately 55%. Besides, Waghorn et al. (1989) found that roughly 32 and 51% of the DM in rumen digesta after eating was retained on 4 and 2 mm sieves, respectively, and that 38 % of the material retained on the 4 mm sieve was over 10 mm length. Recently, Kusmartono et al. (1997), found that the efficiency of

particle breakdown of swallowed ryegrass in the rumen of deer was low, and that this efficiency was considerably reduced when the deer did not ruminate. However, Bosch and Bruining (1995) feeding a relatively dry (average DM content: 58 %) and Gasa et al. (1991) feeding a wetter grass silage (average DM content: 29 %) to dairy cows, found that 26 to 33 % of the DM was retained on a 1.25-1.2 mm sieve, without important changes in rumen particle size distribution during the day. The comparison between different experiments must be viewed with caution since variations in sieve technique (Ulyatt et al., 1986) can markedly influence the results. Additionally, and maybe creating even greater uncertainties, would be to associate or extrapolate results from fresh cut pastures fed in indoor trials to grazing conditions (Ulyatt et al., 1988). It seems that chewing during rumination had a reducing effect only on the proportion of particles between 1.25 and 5 mm length which significantly ($P<0.01$) declined with the allowed grazing time (Figure 2). The proportion of particles retained on the sieve with 5 mm aperture size was not different between treatments which may have been associated with the different location of the particles >5 mm in the rumen. There is a lack of quantitative information on ingestive mastication during grazing (Laca et al., 1994) and on its consequences for digestion and passage.

In Experiment 2, the total, DM, OM and NDF rumen pools after grazing were significantly reduced by the placement of inert material within the rumen but not by the length of starvation before grazing (Table 3). The effects on DM intake of including inert material in the rumen have been extensively studied (Johnson and Combs, 1992; Dado and Allen, 1995; Faverdin et al. 1995) mainly in indoor trials. The reduction in DM intake in our experiment was 197.6 and 165.6 g DM per kg of inert material placed within the rumen for LS and SS cows respectively. This seems to be higher than the mean value (0.59 g DM l-1) derived by Faverdin et al. (1995), but is comparable with the values reported by Baumont et al. (1990) who placed 2 l of polystyrene cubes within the rumen of sheep. However, when we estimated the volume occupied by the 12.5 kg of inert material placed within the rumen (apparent density: 0.625 kg l-1, measured in a graduated cylinder), the estimated reduction in DM intake was about 123 and 103 (g l-1) for LS and SS cows respectively. This is in agreement with the values reported by Dado and Allen (1995) and Johnson and Combs (1991), although contrasts with the lack of response observed by Johnson and Combs (1992). One must bear in mind the fact that we measured the effects of introducing inert rumen bulk in the rumen, only during the first grazing bout where the adaptation capability of the cows (Johnson and Combs, 1992; Faverdin et al., 1993) might have been reduced. The higher, although not significant displacement of rumen volume in LS compared with SS cows, might have be related to the different energetic status of both groups of animals before grazing (Allen, 1996), or to the different placement of the inert material within the rumen, hence, exerting a differential mechanical stimulus over the ruminal wall (Baumont et al., 1990). Differences in rumen digesta density and volume (see below) should also not be dismissed as a possible explanation. It must be noted that the rumen particulate pool sizes of those cows without inert material in the rumen, were not significantly different after grazing from the values before grazing. This similarity would suggest that rumen fill became more important in this experiment than in Experiment 1 but, considering the differences in DM intake and DM intake rate between LS(-) and SS(-) cows (Chilibroste et al., 1997), and the possible associated differences in chewing efficiency during eating, it is hardly possible to estimate the effective effect on filling and/or rumen wall distension produced by the measured rumen pools.

Rumen fermentation products

The smaller pool sizes of SS cows at EMT2 than LS cows at EMT1 (Table 4), might be due to the significant reduction in either the particulate or liquid rumen pool sizes (Table 3), as well as to the reduction in the concentration of the fermentation products, indicating low grazing activity of the SS cows during the night. Low grazing activity and hence low DM intake during the night, has been normally observed in grazing sheep (Penning et al., 1991) and dairy cattle (Rook et al., 1994). Besides, diurnal variation in rumen pool sizes under grazing conditions has been observed by Agabriel et al. (1993) and Thomson et al. (1985)in cattle and sheep respectively. These different ruminal conditions within the day might have an important influence on the effect of supplementation on DM intake and rumen fermentation of grazing dairy cows (Van Vuuren et al., 1986; Rodriguez et al., 1990). Moreover the imbalance on nutrient production and/or absorption may also play an important role in the control of voluntary DM intake in ruminant (Illius and Jessop, 1996).

The increase in the fermentation products of the rumen pool with the allowed grazing time in Experiment 1 (Table 2), may either reflect the higher DM intake of the longer grazing treatments (Chilibroste et al., 1997), or the further availability of the soluble fractions through rumination or both. The similarity between the pools before and after 1 hour of grazing suggest a delay in the availability of the more rapidly fermentable substrate for the microorganisms, probably associated with the low chewing efficiency during eating discussed earlier. The measured fermentation products pool sizes after grazing in Experiment 2 (Table 4) showed a similar trend. Small changes in VFA pool sizes immediately after grazing can be expected with a low efficiency of chewing during eating since one of the important functions of ingestive chewing is to release soluble nutrients (Ulyatt et al., 1986) which are supposed to be immediately and completely fermented. However, after 3.25 hours of grazing, the size of the total as well as the different individuals VFA pools were not significantly different from the values measured at the time of EMT1. This observation supports the hypothesis that the concentration of fermentation products may play a role in the control of voluntary DM intake (Grovum 1987; Van Vuuren 1993) but not as a unique factor (Forbes, 1995), since the cows interrupted the grazing session before reaching the higher levels. It is more probable that a combination of factors rather than any one isolated factor (Mbanya et al., 1993; Forbes 1996) are producing the stimulus which after integration at the central nervous system, will signal the end of the grazing session.

In Experiment 2, the length of starvation before grazing significantly reduced the C3 and total VFA rumen pool after grazing. LS cows showed a significant reduction ($P<0.05$) in the concentration of these metabolites (C3, 13.2 vs 9.2 and total VFA, 85.3 vs 58.2 mmol l-1 for SS and LS cows respectively), which in turn may reflect a lower chewing efficiency during eating by the cows exposed to a larger period of starvation (Greenwood and Demment, 1988). The inclusion of inert material within the rumen significantly reduced the ammonia rumen pool size after grazing, but did not affect the other fermentation products significantly (Table 4). This differential effect on N and non-N compounds might be related to the high nitrogen content and probably high solubility (Van Vuuren et al., 1991) of the forage in this experiment. High ammonia concentrations such as those observed in Experiment 2, have been proposed to affect voluntary DM intake of silages (Gill et al, 1988; Van Os, 1997) and fresh grass (Van Vuuren, 1993). However, despite the differences in grass quality (Chilibroste et

al., 1997), the total as well as the major individual VFA pools were comparable between experiments, except for the longer grazing session in Experiment 1 that exhibited pool sizes somewhat larger than the higher values in Experiment 2. The differences in branched VFA pool sizes between experiments may also reflect the differences in nitrogen content of the grass and nitrogen rumen pool sizes (not presented).

CONCLUSION

The length of the allowed grazing time had a significant effect on DM, OM and NDF rumen pool sizes after grazing, but not on the total rumen pool size. Results from the wet sieve analysis suggest that chewing during eating was relatively low and it seems that cows require a period of rumination to increase DM rumen content and make the soluble fraction available for the rumen microorganisms. In Experiment 1 neither the DM, OM nor NDF rumen pools can be postulated as signalling the end of the grazing session. If it was rumen filling or distention effect, it must have been produced by the total rumen MAT content but a better physical characterization of the rumen content (size, weight and volume of rumen particles) is necessary to be able to properly asses rumen wall distention. The significant and linear increase of different VFA rumen pool sizes with the allowed grazing time, suggest that these fermentation products may be involved in the control of the grazing time in later stages during the day.

The duration of starvation before grazing significantly reduced the rumen DM content, the C3 rumen pool size, and the NGR ratio after grazing. These changes are in agreement with the higher intake rate observed in the long starved treatments and support the idea of a low chew efficiency during eating at the expense of a higher intake rate and a delay in the availability of the more fermentable substrate. The high values of ammonia rumen pool size after grazing in the animals without bulk inert material within the rumen may also be involved in the control of the grazing time of these treatments.

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4 THE USE OF CUMULATIVE GAS PRODUCTION TECHNIQUE TO CHARACTERIZE CHANGES IN THE FERMENTATION CHARACTERISTICS OF RUMEN CONTENTS FOLLOWING VARIABLE PERIODS OF STARVATION AND GRAZING IN DAIRY COWS

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THE USE OF CUMULATIVE GAS PRODUCTION TECHNIQUE TO CHARACTERIZE CHANGES IN THE FERMENTATION CHARACTERISTICS OF RUMEN CONTENTS FOLLOWING VARIABLE PERIODS OF STARVATION AND GRAZING IN DAIRY COWS

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ABSTRACT

The effect of the duration of grazing (Experiment 1) and starvation time, and placement in the rumen of inert bulk material before grazing (Experiment 2), on the rumen content fermentability, was investigated by means of measuring cumulative gas production. In Experiment 1, a comparison was made of four duration of grazing (1, 1.75, 2.50 and 3.25 hours) after overnight starvation. Rumen samples taken from the cows after 1 hour of grazing had higher values of total accumulated gas with less ($p<0.05$) time required to reach the maximum fermentation rate than cows grazed for 3.25 hours. Following grazing, a 7.75 hours starvation period was imposed on the four treatments. The extent of fermentation was significantly lower ($p<0.01$) after starvation than immediately after grazing (49.7 vs. 60.8 % of incubated dry matter), respectively. Experiment 2 consisted of a factorial combination of two duration of starvation before grazing (16.5 [LS] and 2.5 [SS] hours) with the presence or absence in the rumen of 12.5 kg of a synthetic indigestible material. Before grazing the total accumulated gas production was less ($p<0.05$) for the LS than for the SS cows. After the grazing session, the total gas of rumen samples from the LS cows were significantly higher ($p<0.05$) than for the SS cows. This was in agreement with the observed higher DM intake during grazing and DM rumen pools after grazing in LS cows. For both starvation periods, the presence of inert rumen bulk led to a higher total gas, a shorter half time and less DM left unfermented. The measurement of fermentation kinetics by cumulative gas production was suitable to detect changes in rumen content fermentation patterns due to the clearance of material from the rumen (effect of starvation) or DM intake during the grazing sessions.

Keywords: gas production, rumen contents, starvation, grazing, fermentation, rumen fill, cattle.

INTRODUCTION

The measurement of cumulative gas production (GP) has been proposed as a simple and accurate *in vitro* method to study the fermentability of feeds (Beuvink et al., 1992; Pell and Schofield 1993; Theodorou *et al.* 1994). Good correlation between GP parameters and *in vivo* measurements such as dry matter intake (Blümmel and Ørskov, 1993; Blümmel and Becker, 1997) or *in vivo* digestibility (Blümmel and Ørskov, 1993; Khazaal *et al.*, 1993) have been found.

The importance of the rate and extent of rumen fermentation for feed characterisation has been extensively recognised (i.e.:. Noceck and Tamminga, 1991), and been progressively incorporated into the standard systems for feed evaluation (i.e.: NRC, 1985; Sniffen *et al.*, 1992; Tamminga *et al.*, 1994). Also, attempts have been made (Beuvink *et al., 1993;* Blümmel and □rskov, 1993; Khazaal *et al.*, 1993; Chilibroste *et al.*, 1998b) to correlate

cumulative GP and the nylon bag technique (Mehrez and □rskov, 1977), which has previously been widely used as a standard method to assess the degradation kinetics of different feeds fractions. The validity of such a comparison has been questioned (Williams, personal communication), since the two methods measure two completely different biological processes (accumulation of end-products, versus loss of small particles) and different results must be expected. Such a comparison can only be made if they are both being related to *in vivo* parameters.

New automated equipment has been developed to measure gas production (Cone *et al.*, 1994; Davies *et al.*, 1995) so that more accurate gas production profiles are being generated, in which different underlying processes can be identified (Groot *et al.*, 1996) and the fermentative behaviour of soluble fractions isolated and described (Cone *et al.*, 1997; Schofield and Pell, 1995; Stefanon *et al.*, 1996). These latest developments make GP a promising technique to improve our understanding of rumen fermentation kinetics.

In this paper we report the use of cumulative GP to characterise changes in the fermentation patterns of rumen contents after different treatments involving duration of starvation and grazing period with or without artificial rumen bulk (Chilibroste *et al.*, 1997). So far no information has previously been reported concerning the fermentability of rumen contents according to cumulative GP. An analysis has been made of the relationships between kinetic GP parameters and the chemical composition of rumen contents.

MATERIAL AND METHODS

Treatments and design

Two *in vivo* experiments were carried out in June (Experiment 1) and September (Experiment 2) 1995, at the experimental farm "De Ossekampen" of the Wageningen Agricultural University, The Netherlands. In both experiments, four cows were randomised across four treatments during four measurement periods, according to a balanced 4*4 Latin square design (Steel and Torrie, 1980). Treatments in Experiment 1 concerned the duration of the grazing session: 1, 1.75, 2.50 and 3.25 hours of grazing after overnight starvation. After the grazing session a new period of starvation of 7.5 hours was imposed. Treatment effects on fermentability of rumen content immediately after grazing and after 7.5 hours of starvation were tested using cumulative gas

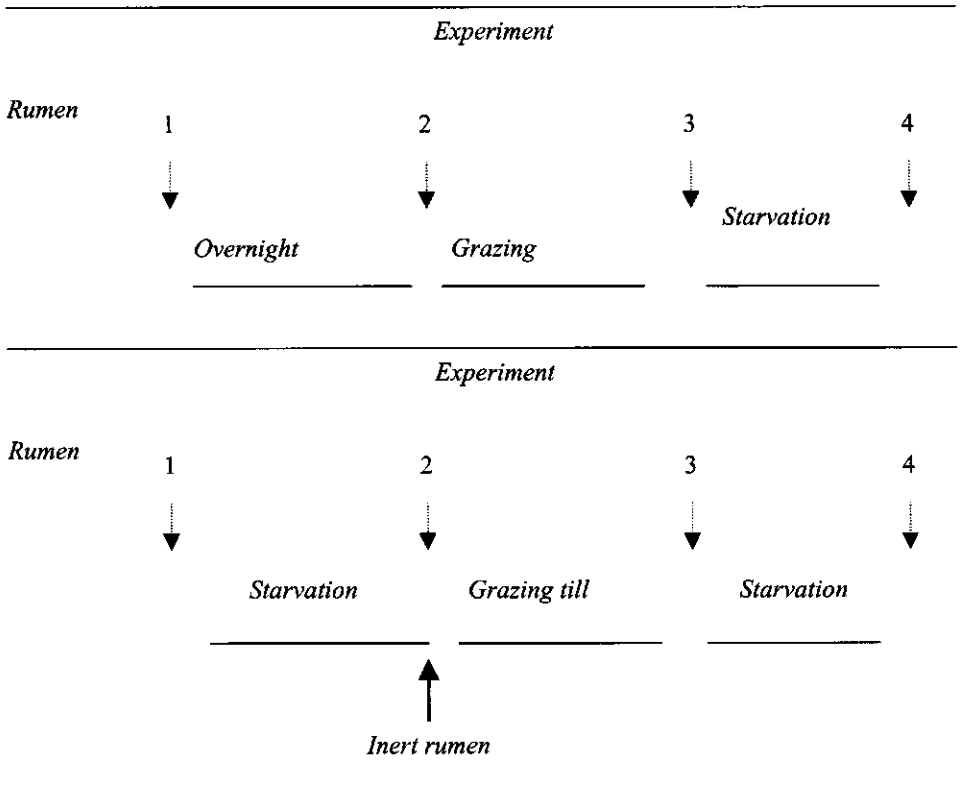


Figure 1. Diagrammatic representation of the procedure followed in Experiments 1 and 2.

Table 1. Distribution of samples through the gas runs.

Experiment	Gas Run	Emptying
1	A	3 and 4
1	B	1 and 2
2		1 and 4
2	C	2 and 3

Table 2. Measured cumulative gas production (G, ml g^{-1} OM incubated), estimated gas production (A, ml g^{-1} OM incubated), yield of gas (Y_{OM}, ml g^{-1} OM fermented), time for half of gas production (C, h), sharpness of the curves (B), and sample dry matter left (DML, %) after the incubation of control samples on gas runs A, B and C.

	Parameters					
Gas run	G	A	Y_{OM}	B	C	DML
A	368.8[a]	376.3[a]	363.3[a]	1.56[ab]	13.4[a]	13.2[a]
B	328.3[b]	330.6[b]	372.8[a]	1.64[a]	13.2[a]	18.1[a]
C	318.2[b]	327.5[b]	333.1[a]	1.48[b]	15.3[a]	16.6[a]
Mean	340.3	346.4	355.2	1.57	13.8	15.4
SEM	11.13	12.14	20.85	0.076	0.51	3.52

Means in the same row with different superscripts differ significantly ($p<0.05$); SEM, pooled standard error.

production measurements. Experiment 2 consisted of a factorial combination of two duration of starvation periods before grazing (SS=2.5 and LS=16.5 h, respectively), and the presence (+) or absence (-) of 12.5 kg of a synthetic indigestible material (Shell polystyrene, particle size = 4x3 mm, specific density, 1.03 g ml^{-1}) in the rumen. Hence, four treatments were compared: SS(+), SS(-), LS(+) and LS(-). The effect of starvation time, addition of inert rumen bulk, and their interaction on rumen content fermentability were tested. Correlation analysis between GP parameters and rumen chemical composition and rumen pools sizes, and rumen content clearance rates, were performed using the complete data set generated in both experiments. All the statistical analyses were performed using the GLM procedure of SAS (SAS Institute INC., 1989).

Animals and experimental periods

Four lactating dairy cows were used which had been surgically prepared with a large rumen cannula (10 cm id., Bar Diamond Inc., Parma, Idaho, USA). A diagrammatic representation of the sequence followed in both experiments is shown in Figure 1. The process of evacuation, weighing, sampling and return of the whole rumen contents to the cows, will be referred to in this paper as emptying. The sequence of emptying was numbered from 1 to 4: emptying 1 (EMT_1) was done before the first starvation period; emptying 2 (EMT_2) the morning following starvation and before grazing; emptying 3 (EMT_3) immediately after the experimental grazing session; and emptying 4 (EMT_4) after the period of starvation that followed EMT_3 (Figure 1). Detailed descriptions of animal and sward management, the rumen emptying procedure, rumen pool sizes, and fermentation and clearance rate determinations have been published elsewhere (Chilibroste *et al.*, 1997; Chilibroste *et al.*, 1998a). Note that in Experiment 1 the treatments were predetermined allowed grazing times, while in Experiment 2 the treatments were applied before grazing (Figure 1) and grazing time was one of the response variables measured. Results and discussion are focussed on emptying 3 and 4 for Experiment 1 and emptying 2 and 3 for Experiment 2. The correlation analysis was performed with the complete data set (emptying 1 to 4 for both experiments).

Gas production measurements

The rumen content sampling procedure has been described in detail elsewhere (Chilibroste *et al.*, 1998a). In summary, all the digesta that could be removed by hand (MAT) were placed in an insulated container and the remaining material (BAILABLE) collected with a plastic bottle. The MAT material was weighed, mixed by hand, and two sub-samples (approximately 400 g each) taken. The BAILABLE material was added to the MAT sub-samples in the same proportion (wet basis), as had been present in the rumen. The composed rumen samples (BAILABLE + MAT) were kept at -20 °C and analysed for DM, ash, Kjeldahl N, Neutral Detergent Fibre (NDF) and Acid Detergent Lignin (ADL). Dry matter content was determined by freeze-drying to constant weight, ash in an oven at 550 °C and N by the Kjeldahl method with $CuSO_4$ as the catalyst. Neutral detergent fiber and ADL were determined according to the method of Goering and Van Soest (1970).

The fermentative characteristics of the composed rumen samples were assessed according to the procedure reported by Theodorou *et al.* (1994) as modified by Williams *et al.* (1995). In this case, 24 readings were done over 144 h, with readings occurring more frequently in the early stages of fermentation. The inoculum comprised rumen fluid from three sheep fed a constant diet of medium-quality hay, and was collected following overnight starvation. Each rumen sample was run in quadruple (approximately 0.5 g of substrate per incubation). Because the large number of samples to be incubated they were split into three different gas runs as described in Table 1. To allow for between gas runs differences (e.g. variation in rumen fluid), a correction factor was introduced by use of a standard substrate (ryegrass hay) as the reference sample for all the runs. Gas for this standard was measured alongside the rumen samples, the curve was fitted, and the correction factor for each gas run was calculated by comparing the parameters of the
standard sample with the mean value of all gas runs. When comparisons between experiments and emptying were required (i.e. correlation analysis) the correction factor for each parameter was applied to the different gas runs.

The cumulative gas production profiles were fitted to the model as described by Groot *et al.* (1996):

$$G = \sum_{i=1}^{n} \frac{A_i}{1 + \frac{C_i}{t}^{B_i}}$$

where i indicates the number of phases in the profile (i = 1, n), G (ml g^{-1} OM) is the cumulative gas production per g of OM incubated at time t after incubation, A_i (ml g^{-1} OM) represents the asymptotic gas production, C_i is the time after incubation at which half of A_i has been produced and B_i is a constant determining the sharpness of the curve. The fractional rate of substrate digestion and the time (t_{Rm}) at which the maximum digestion rate occur (R_m),

$$t_{R_m} = C(B-1)^{1/B} \quad ;$$

$$R_m = \frac{B t_{R_m}^{B-1}}{C^B + t_{R_m}^{B}}$$

can be calculated from the parameters B and C as follows:

Note that for these calculations a fixed linear relationship between substrate fermentation and gas production must be assumed. The model parameters were estimated by using the non-linear curve-fitting program NLREG (Sherrod, 1995)

To fit mono-, bi- and tri-phasic models the second fitting approach of Groot et al. (1996) was used. The significance of individual model parameters was assessed by testing their t-value (parameter value/standard error) against tdf ($p<0.05$; df= degree of freedom). The significance of improvement of fit when the number of phases in the model was increased from i to i+1 was determined according to Zwietering et al. (1990).

RESULTS

All the GP parameter reported were estimated with a monophasic model except otherwise indicated.

The estimated GP parameters for the control samples between gas runs are shown in Table 2. The A gas run exhibited significantly higher values of gas production per g OM incubated, for both measured and estimated model parameters. However, when the gas production was expressed in terms of ml gas per g OM disappeared from the bottle (Y_{OM}, Table 2), the differences between gas runs were not significant. The amount of DM disappeared from the bottles after 144 hours of incubations tended to be higher in the gas run A although not significant.

The effect of different period of grazing after overnight starvation (Experiment 1) on fermentation characteristics of rumen samples is shown in Table 3. The amount of gas produced per g OM incubated diminished as the grazing session progressed. Although the half time appeared to have increased with increased duration of grazing period, these differences were not significant. After the grazing treatments, and the application of a period of starvation, the trend was reversed: the cows which had grazed longer had rumen contents which produced more gas per g of OM incubated, and lower values of DM left after incubation (Table 3). As expected, the extent of fermentation was significantly lower ($p<0.01$) after starvation than immediately after grazing (49.7 vs. 60.8 %).

The effect of duration of starvation period before grazing (Experiment 2), on rumen samples gas production parameters is shown in Table 4. The measured cumulative gas production was significantly different between duration of starvation before grazing. The time required to produce half of the total gas was significantly lower in SS than in LS rumen samples. After grazing however, the cumulative gas production of rumen samples coming from the cow starved longer (LS, Table 4) were significantly higher than the rumen samples of SS cows. In both cases, the presence of inert rumen bulk led to a higher cumulative gas production, a shorter half-time and less DM remaining after incubation (Table 4).

Table 3. Measured cumulative gas production (G, ml g^{-1} OM incubated), estimated gas production (A, ml g^{-1} OM incubated), yield of gas (Y_{OM}, ml g^{-1} OM fermented), time for half of gas production (C, h), sharpness of the curves (B), and sample dry matter left (DML, %) after the incubation of rumen samples of cows with different allowed grazing times and starved after grazing for 7.75 hours. Experiment 1.

Variable	Emptying after grazing				SEM	Emptying after starvation				SEM
Grazing length, h	1.00	1.75	2.50	3.25		1.00	1.75	2.50	3.25	
G	271.5^{a}	268.5^{a}	263.7^{ab}	255.6^{b}	13.73	194.9^{c}	203.0^{bc}	213.1^{ab}	214.9^{a}	11.80
A	285.2^{a}	285.9^{a}	277.2^{ab}	269.3^{b}	16.18	215.9^{c}	219.6^{bc}	229.4^{ab}	232.1^{a}	12.98
B	1.56	1.57	1.59	1.60	0.056	1.59^{b}	1.67^{a}	1.67^{a}	1.66^{a}	0.07
C	21.4	22.4	23.0	23.6	2.932	34.4	31.6	30.8	30.5	2.28
DML	38.1	39.9	39.5	39.2	2.547	51.9^{a}	50.2^{a}	49.6^{b}	49.2^{b}	1.83
t_{Rm} h	14.7^{b}	16.0^{ab}	16.3^{ab}	16.6^{a}	1.750	24.4	24.8	24.0	23.9	2.55
Rm % h^{-1}	3.88	3.55	3.77	3.55	0.39	2.42	2.73	2.79	2.82	0.21

Means in the same row with different superscriptsdiffer significantly ($p<0.05$). t_{Rm}, time at which maximum degradation rate happened; R_m, maximum degradation rate.

Table 4. Measured cumulative gas production (G, ml g^{-1} OM incubated), estimated gas production (A, ml g^{-1} OM incubated), yield of gas (Y_{OM}, ml g^{-1} OM fermented), time for half of gas production (C, h), sharpness of the curves (B), and sample dry matter left (DML, %) after the incubation of rumen samples of cows long (LS) or short (SS) starved with the addition (+) or not (-) of inert rumen bulk beforegrazing. Experiment 2

Variable	Emptying before grazing			Sig.	Emptying after grazing					Significance		
	LS	SS	SEM		LS (+)	LS (-)	SS (+)	SS (-)	SEM	Starv	Bag	Int.
G	134.7	143.3	13.77	***	204.2	197.7	173.3	164.2	12.70	***	**	NS
A	155.2	159.7	13.10	NS	211.7	204.4	184.2	174.6	13.97	***	**	NS
B	1.47	1.54	0.08	***	1.61	1.69	1.61	1.59	0.08	***	**	NS
C	39.9	34.6	5.21	***	21.5	23.2	26.4	27.2	2.15	***	**	NS
DML	59.2	57.9	3.11	NS	43.6	45.1	51.7	52.5	2.20	***	**	NS

Sig., significance; Starv, starvation; Bag, inert rumen bulk; Int, interaction; ***, $p<0.05$; NS, no significant. Other symbols and units see Table 3.

The correlations between the estimated GP parameters and the chemical compositions and rumen pool sizes of rumen contents are shown in Table 5. The asymptotic cumulative gas production was negatively correlated with the N and ADL content of the rumen samples and with the total ADL rumen pool size. The yield of gas (Y_{OM}, ml g^{-1} OM disappeared) followed the same trend, although the magnitude of the association with the ADL content of the rumen samples seemed to be small. The amount of DM left after incubation was significantly correlated only with the ADL content of the rumen samples.
For the rumen samples taken after 1 hour of grazing a mono-, di and tri- phasic model was fitted since more than one fermentation pattern was suspected. The estimated parameters are shown in Table 6. There was no improvement in the goodness of fit as reflected by the F-test, despite the reduction in the error sum square. The added parameters A_2 and B_2 were not significantly different from 0. For the three-phase model, the reduction in error sum squares compared with the mono- phasic model tended to be significant (F-test; $p<0.1$), but the high standard errors associated with B_1 and C_1 suggested over- parameterisation of the model. Regarding the distribution of residuals (Figure 2), the two-phase model improved the fitting in the first hours of fermentation and then followed the same trend compared with the mono-phasic model. Adding a third phase did improve residual distribution during the firs 30-40 hours and then followed the same trend as the previous models (Figure 2).

DISCUSSION

Differences between gas runs

Although the GP procedure was the same between the different gas runs, differences of 6 to 8 % were found in the cumulative gas production of the control samples (Table 2). These differences are similar to those reported by Cone *et al.* (1996) who found day-to-day variation of the same magnitude between gas run series, and were corrected for by including the standard sample in each run.

Experiment 1

Rumen samples taken from the cows after 1 hour of grazing had significantly higher values of cumulative gas production and lower t_{Rm} than the cows with longer grazing times (Table 3). This was an unexpected result, since the cows in the shorter grazing session had significantly less DM intake (3.52 vs. 4.35 kg) and a smaller rumen pool size (7.86 vs. 9.17 kg) than the cows in the longer grazing session. However, the ingestive behaviour exhibited by the cows, differed over the grazing time. The cows during the first hour of grazing exhibited a very high intake rate (0.61 percent of the body weight per hour) with a large bite mass (0.97 g per bite) (Chilibroste *et al.*, 1997), though probably at the expense of lower chewing efficiency during eating. Further determinations on the rumen content samples (wet sieve analysis) and rumen liquid (volatile fatty acid and ammonia concentration) offered additional evidence (Chilibroste *et al*, 1998a) suggesting that for the shorter grazing session the soluble fraction of the material just ingested had been only partially released for the micro-organisms. Groot *et al.* (1996) and Schofield *et al.* (1994) have suggested the use of different logistic multi-phasic models to

detect different pools with their associated fermentation patterns. To test whether or not different fermentation patter were present in the rumen samples after 1 hour of grazing a mono-, di and tri- phasic models were fitted. The estimated parameter for these models are shown in Table 6. There was no significant improvement in the goodness of fit as reflected by the F-test, despite the reduction in the error sum square. Regarding the distribution of residuals (Figure 2), the two-phase model improved the fitting in the first hours of fermentation and then followed the same trend compared with the mono- phasic model. Adding a third phase did improve residual distribution during the first 30-40 hours afterwhat the tree phase model followed the same trend as the other models. Despite differences in the substrates used by Cone *et al.* (1997), Schofield and Pell (1995) and ourselves, two additional restrictions could have inhibited the identification of early phases with our approach: the high dilution of the inoculum in our system and the use of manual reading system that only allowed four points in the first 10 hours of incubation (Chilibroste *et al.*, 1998b).

After 7.75 hours of starvation, the cumulative gas production per g OM incubated was significantly lower than immediately after grazing ($p<0.01$) for all the treatments. Besides, the time required to produce half of the total cumulative gas production and the amount of DM left after 144 hours of incubation were significantly higher ($p<0.01$) after starvation. These differences between the fermentation parameters of the rumen contents immediately after grazing and after 7.75 hours of starvation, were most probably a reflection of the clearance of material (degradation plus passage) of the different fractions from the rumen. The more slowly degradable fractions remained in the rumen during starvation, and the poor fermentation characteristics of this material were well reflected by the GP parameters. The differences between treatments observed at the end of the starvation time (G, A: 3.25 hours of grazing > 1 hour of grazing; C, DML: 3.25 hours of grazing < 1 hour of grazing; Table 3) was in agreement with the higher DM intake during the grazing session and the observed DM pool sizes after grazing (Chilibroste *et al.*, 1997). When the cows in the shorter grazing period (Table 3) had the first rumination bout after grazing, the soluble nutrients were probably released (Ulyatt *et al.*, 1986), and were immediately and completely digested. As the DM intake during this grazing session was the lowest, the remaining DM rumen pool after this first rumination bout, must have had a higher proportion of indigestible fractions.

Table 5. Correlation between Gas Productions (GP) parameters and rumen content chemical composition and rumen pool sizes. Experiments 1 and 2.

		Rumen chemical composition g kg. DM^{-1}			Rumen pool sizes (P) Kg.		
GP parameters		N	NDF	ADL	N-P	NDF-P	ADL-P
A	r	-0.46	0.009	-0.736	0.001	0.132	-0.285
	p	<0.001	0.928	<0.001	0.989	0.212	0.006
	No	96	90	98	96	96	89
B	r	-0.033	0.262	-0.197	0.079	0.050	-0.047
	p	0.747	0.012	0.063	0.441	0.636	0.656
	No	92	90	89	96	90	89
C	r	-0.053	0.525	0.237	-0.541	-0.561	-0.352
	p	0.605	<0.001	0.025	<0.001	<0.001	<0.001
	No	98	90	90	96	90	89
Yom	r	-0.326	-0.043	-0.487	0.098	0.195	-0.078
	p	0.0012	0.685	<0.001	0.339	0.064	0.465
	No	96	98	90	96	90	89
DML	r	0.061	-0.018	0.312	-0.145	-0.177	0.005
	p	0.548	0.866	0.0029	0.1578	0.094	0.603
	No	96	90	89	96	96	89

G, measured cumulative gas production (ml g^{-1} OM incubated); A, estimated gas production (ml g^{-1} OM incubated); Y_{OM}, yield of gas (ml g^{-1} OM fermented); C, time for half of gas production (h); B, sharpness of the curves; DML, sample dry matter left (DML, %); r, Pearson correlation coefficient; p, probability ≠ 0; No, number of observations.

Table 6. Estimated values of gas production parameters for rumen samples of cows after the first hour of grazing (Experiment 1) in mono, di- and triphase models

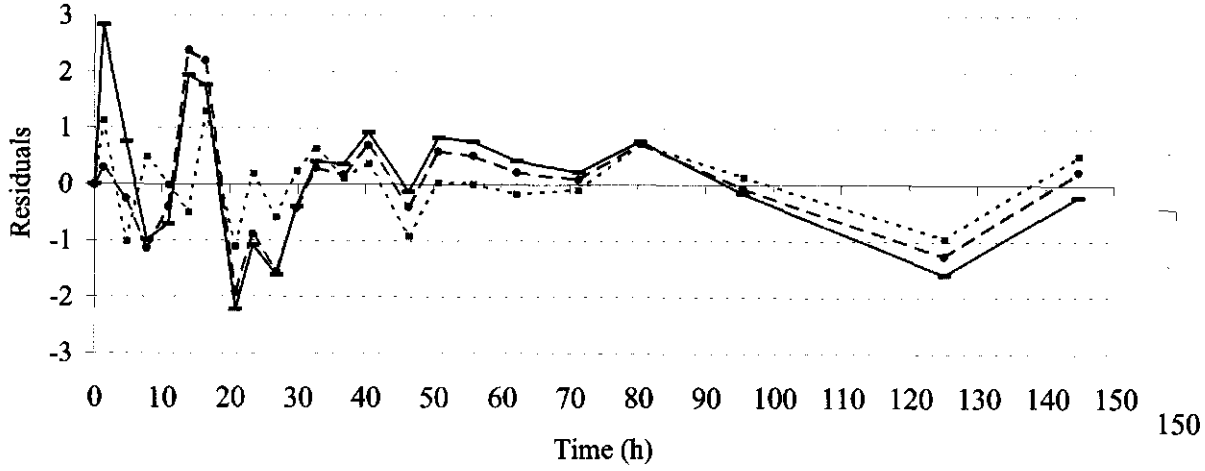
	One-phase		Two-phases		Three-phases	
Parameter	Value	Prob. (*t*)	Value	Prob. (*t*)	Value	Prob. (*t*)
A_1	285.9	<0.01	281.5	<0.01	11.5	<0.01
A_2			3.8	0.16	217.8	<0.01
A_3					57.4	0.17
B_1	1.52	<0.01	1.51	<0.01	127.7	1
B_2			11.8	0.43	1.32	<0.01
B_3					2.73	<0.01
C_1	21.0	<0.01	20.7	<0.01	10.9	1
C_2			31.9	<0.01	20.3	<0.01
C_3					27.2	<0.01
DW	1.57		1.97		3.02	
SSE	31.61		24.15		9.26	
F			1.573 NS		2.35 p<0.1	

A, estimated gas production (ml g^{-1} OM incubated); C, time for half of gas production (h); B, sharpness of the curves. Subscript i represent phase number. DW, Durbin-Watson coefficient for autocorrelation; F-test indicates the improvement of the fit after adding two o three phases over one phase fitting; SSE, sum square error of the model. Prob. (*t*),*t-test* of the parameters significantly different from 0.

Experiment 2

Despite the large differences in DM rumen pool size before grazing (4.8 vs. 8.3 kg for LS and SS, respectively), the differences in GP parameters were smaller though still significant. These small differences in cumulative gas production per g of OM incubated were probably a reflection of the predominance of fibre in the rumen for both duration of starvation period (Schofield and Pell;1994) and the low amounts of soluble fractions present. This means that the grazing activity and hence the DM intake during the night might had been low (Rook *et al.*, 1994) in those animals not starved overnight. The cows starved for longer periods had a higher dry matter intake associated with longer grazing sessions (Chilibroste *et al.*, 1997), but similar DM rumen content pool sizes (Chilibroste et al., 1998a) than the cows starved for shorter periods. The significantly higher cumulative gas production and extent of fermentation, and the shorter half-time observed in the LS animals emptied after grazing (Table 4), might be related to the higher DM intake and higher proportion of new material (just ingested) over the amount of material present before grazing than for SS animals. It must be emphasised that in Experiment 2 the cows were allowed to graze till voluntary stop, while in Experiment 1 the duration of grazing was the applied treatment. That means that in Experiment 2 rumination bouts were not present during the grazing session and commutation of particles occurred mainly during ingestive mastication.

Cumulative gas production was significantly correlated with the ADL content of the rumen samples but not with the NDF content (Table 5). However NDF was positively correlated with the time required to produce half of the total gas, giving an indication of the sharpness of the gas production profile. The amount of gas produced per g OM fermented (Yield OM, Table 5) was negatively correlated with the N content of the rumen samples. The N content of the samples exerted a negative effect on the total cumulative gas production as was observed by Chilibroste *et al.* (1998b) for grass samples. Menke and Steingass (1988) have suggested that CO_2 can be captured by NH_4 and from NH_4HCO_3 , leading to an underestimation of the amount of gas produced. Besides, Blümmel and Becker (1997) found that more gas was produced from 200 mg NDF than from 200 mg whole roughage and suggested the efficiency with which the fermentable material is incorporated into microbial cells as an explanation for the higher gas volume from NDF.



—, continuous line = monophasic model; •, dashed line = biphasic model; □, dotted line = triphasic model.

Figure 2. Residuals distribution after fitting a mono-, di and tri- phasic model to the observed gas production profile of rumen samples taken after 1 hour of grazing. Experiment 1.

CONCLUSIONS

The measurement of fermentation kinetics by cumulative gas production was suitable to detect changes in rumen content fermentation patterns due to the clearance of material from the rumen (effect of starvation) or DM intake during the grazing sessions. The GP parameters were more related to the rumen content chemical composition than to the rumen pool size. The use of GP technique to characterise nutrient availability to ruminal micro-organism require additional research.

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5 EFFECT OF DURATION OF REGROWTH OF RYEGRASS (*LOLIUM PERENNE*) SWARDS ON: 1. GRAZING BEHAVIOR, DRY MATTER INTAKE AND RUMEN FILL OF LACTATING DAIRY COWS

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ABSTRACT

The relative importance of duration of sward regrowth and rumen fill on the control of grazing time during the first grazing session of the day were studied. Four lactating dairy cows were allowed to graze ryegrass (*Lolium Perenne*) swards, which had been allowed 5 different regrowth periods after mowing (6, 9, 16, 22 and 30 d). The cows were allowed to graze until they stopped voluntarily. Before and after grazing the rumen content was evacuated, weighed, sampled and returned to the animals. Grazing time did not follow a significant trend with period of regrowth, and exhibited a profound discontinuity between experimental days 6 and 16. When the pasture had 9 days of regrowth the cows attempted to compensate for a reduced bite mass and intake rate by increasing grazing time. Neither intake rate nor bite mass exhibited a significant trend with period of regrowth. The lowest and highest values of intake rate and bite mass were observed on regrowth days 9 and 30 respectively. Bite rate did not change significantly with duration of regrowth with cows exhibiting high rates of biting for all the sward conditions. Both the total and DM rumen pools sizes after grazing increased significantly with the days of regrowth ($P < 0.01$). Nevertheless the cows always stopped grazing before a maximum rumen capacity was reached. Dry matter rumen pool size is unlikely to be a good indicator of short-term rumen fill.
(**Key words:** grazing behavior, dry matter intake, rumen fill, grass)

Abbreviation Key: GT = grazing time, **MBR** = mean bite rate, **DMRP** = dry matter rumen pool.

INTRODUCTION

The mechanisms that control DMI in ruminants have received much attention since feed intake is the predominant factor determining animal performance. If DMI is seen as the summation of individual discrete meals (9), understanding what causes an animal to start and stop eating would lead to a better understanding and prediction of daily DMI. Nevertheless, different factors may predominate in ending meals during a 24-h period (14). In dairy cows fed forages, a physical limitation has been proposed as the main constraint to obtaining higher DMI (28). However there are at least two feeding situations where the theory of physical regulation of DMI fails to explain the observed DMI: dairy cows fed with silage (15) and those fed with high quality fresh forage (38). Under grazing, sward condition might appear as the first constraint limiting DMI (e.g., 10, 17). Increasing grazing time is the main response mechanism exhibited by cows to cope either with changes in their physiological status (4) or with restrictive sward conditions (12, 32). For lactating dairy cows two major grazing bouts have been observed: one in the morning and the largest in the afternoon (12, 32). Nevertheless little progress has been made to determine the main factors controlling grazing time (20).

In this paper we discuss the relative importance of duration of regrowth and DM rumen fill on the control of grazing time and DMI during the first grazing bout after a.m. milking. In a companion paper we discuss the relative importance of rumen fermentation end products pool

sizes and rumen environmental variables, such as pH and osmotic pressure over the same response variables.

MATERIAL AND METHODS

General Procedure

The experiment was carried out from May 13th to June 30th of 1996 at the experimental farm "De Ossekampen" of the Wageningen Agricultural University. Four lactating Holstein-Friesian cows previously fitted with a large rumen cannula (10 cm Bar Diamond Lane, Idaho, USA) in the dorsal rumen sac were used. At the start of the experiments the cows weighed 532.7 ± 55 kg and produced 27.5 ± 5.1 kg/d milk (X ± SE). The cows were milked twice daily at 0600 and 1600 h and ten days before the experiments started they were trained to be led and graze whilst tethered. During the maesurement days the cows grazed individually, tethered within a circular plot. Between measurement days cows grazed in a contiguous plot of ryegrass. During the whole experiment the cows did not receive any supplementary feed. The experimental plot comprised 2.5 hectares of ryegrass (*Lolium perenne)* sown in 1989. In the spring of 1996 the pasture was fertilized with 139 kg of nitrogen and with 24 kg of phosphorous per hectare and was not grazed or mown before the start of the experiment.

The treatments comprised 6 different periods of regrowth. At day 0 (May 13) the whole pasture was cut with a mowing machine (cutting height 4-cm) and the cuttings removed. On days 6, 9, 13, 16, 22 and 30 after cutting (referred to as measurement days) the cows grazed whilst tethered (4) during their first grazing bout of the day. The general procedure for an experimental day is shown in Figure 1. After morning milking, rumen evacuation of the four cows was conducted between 9.00 and 11.00 h a.m. At 11.00 h the four cows were placed in their respective grazing plots and allowed to graze until they stopped voluntarily. Immediately after grazing, each cow was removed to the barn and the rumen was evacuated again. After replacement of this second rumen evacuation, the cows were starved till the next morning when a third rumen evacuation was carried out. Following this third rumen evacuation the cows were allowed to graze freely in a contiguous plot. The sward mass offered per cow and per grazing session on each measurement day was 19.8 ± 4.2 kg DM, measured 2.5 cm above soil surface. It was obtained by adjusting the length of the rope (the radius of the circle) that restrained the movements of the cows in the plot. The grazing plots were marked and sampled the day before each experimental day. Individual milk production was recorded during four consecutive milking with the last being the morning belonging to the measurement day.

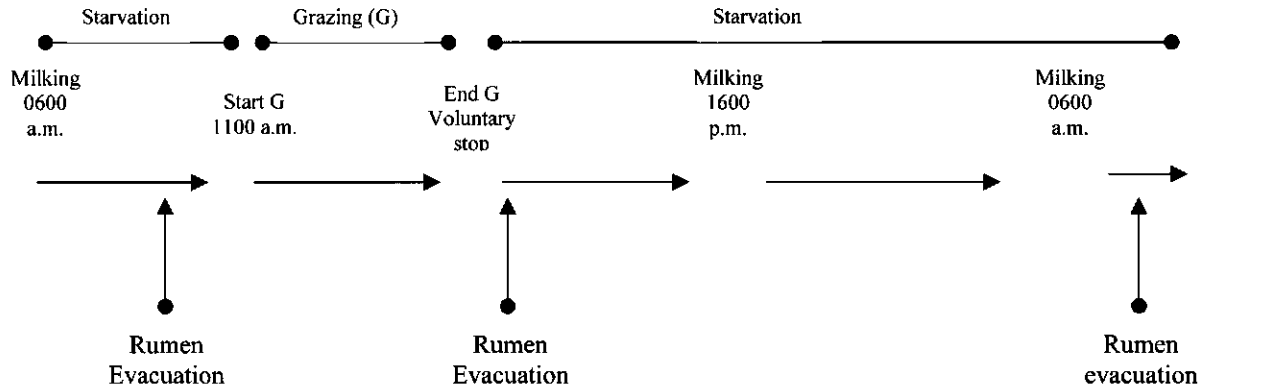

Figure 1. General procedure during an experimental day

Pasture Determinations

The sward mass available before and after grazing was estimated using the double sampling technique (27). Five quadrats (0.5 * 0.5 m) of contrasting sward mass and height were selected per plot. Sward height measured with a plate meter (weight: 350 g, diameter: 0.5 m) and sward mass over 2.5 cm were determined for each square. The square was fitted with 6 parallel guides to keep the cutting height as uniform as possible. A calibration was calculated by regressing sward mass against sward height. Around 30 additional measurements of sward height were made at 2 meter intervals in 6 straight parallel lines in each plot. Mean sward mass for the plot was estimated from the mean of these values and using the regression. The mean, median, minimum, maximum and quartiles 25 and 75 % characterized sward height distribution. The height range lying between the upper and lower quartiles was used to indicate the heterogeneity of the swards before and after grazing.

Cut grass samples were collected into plastic bags, weighed fresh, dried at 60 °C to constant weight and the dry weight (after acclimatization) registered as air DM. Air DM forage samples were analyzed for DM, ash, N and NDF content. Dry matter was determined by drying the samples to constant weight in an oven at 103 ° C, ash by combustion in a muffle at 550 ° C and N by the kjeldahl method with $CuSO_4$ as a catalyst. Neutral detergent fiber was measured according to the method of Van Soest et al. (37).

Animal Grazing Behavior

Grazing time (**GT**) was recorded as the time elapsed from the moment the cows were placed on the experimental plot until grazing ceased. Grazing was considered to have finished when a cow had met one of two criteria: either she had lain down after an active grazing period (1) or 15 minutes had passed without any biting activity (2). In most instances cows met the first criterion. A trained observer continuously recorded grazing activity of the four cows to measure periods of non-grazing activity shorter than 15 min but larger than 1 min. Biting and searching with the head down were considered as grazing activities. Other activities (including urination and defecation) were considered as not grazing. Eating time (13) was calculated as GT minus the minutes without biting activity. Bite rate was recorded by the same observer over a period of 1 minute every ten minutes (18) for each cow. The sound when the cows severed the herbage was easily audible and chosen as the criterion to define and count bites. Mean bite rate (**MBR**) was calculated over all recorded minutes (GT), and adjusted bite rate considering only those recorded minutes during which bites were registered. The total number of bites was calculated as the product of GT and MBR.

Rumen Contents

The rumen evacuation procedure was conducted as described by Chilibroste et al. (3, 4). To improve the collection of liquid that drained while the evacuation of the solid fraction was

taking place, a steel latticework with 4 legs of 15 cm each was placed within the insulated collection container. The steel latticework and the sides of the container were covered with a double layer of lace curtain. The solid rumen contents were removed by hand and placed in insulated containers that prevented the material cooling rapidly. Solid material was weighed, mixed by hand in the insulated containers and two subsamples taken (approximately 400 g each). Material not removable by hand was collected with a plastic bottle and sieved (pore size, 0.04 mm^2) into a 40-L container. The liquid drained in the bottom of the insulated container was also sieved through a pore size of 0.04 mm^2 into the liquid container. Then the liquid fraction was weighed, sampled (approximately 1 L) and returned to the rumen, so that the rumen remained completely empty for only two or three minutes. Liquid was added to the solid subsamples in the same proportion (wet basis) as they were presents in the rumen. The reconstituted samples (solid + liquid) were kept at -20 ° C until DM content was determined by freeze drying to constant weight. Total rumen pool size was calculated as the sum of solid and liquid fractions. Dry matter rumen pool **(DMRP)** size was calculated as the product of the total weight of rumen contents and their DM content.

Dry Matter Intake

The DMI was estimated from the changes in DMRP as follows:

$$DMI = (RPAG - RPBG) + RPBG\,(1 - \exp^{(-k_{cl} \times GT)}) + CNGI$$

where

RPAG = DMRP after grazing (kg),
RPBG = DMRP before grazing (kg),
k_{cl} = clearance rate (h^{-1}) of RPBG during the grazing session,
CNGI = clearance of rumen DM ingested during the grazing session (kg), and
GT = grazing time (h).

To calculate CNGI the following assumptions were made: a uniform pattern of ingestion through the grazing session and a mean residence time of the particles ingested of 0.5 GT. The calculations were conducted as follows:

$$CNGI = [(RPAG - RPBG) + RPBG\,(1 - \exp^{(-k_{cl} \times GT)})] \times (1 - \exp^{(-k_{cl} \times 0.5\,GT)})$$

Rumen DM clearance rate (k_{cl}) was estimated over the starvation period following the grazing session (Figures 1), assuming a first-order kinetics (31) with one pool being cleared at a constant fractional rate.

Statistical Analysis

Analysis of a compound symmetric variance structure was tested using the MIXED procedure with cow as a repeated subject (34). As the compound symmetric structure was not significant, a general linear model was applied. Linear and quadratic effects of days of regrowth on sward chemical composition, grazing behavior, DMI, and DMRP sizes were estimated using the GLM procedure of SAS (34). Cows were treated as blocks and heterogeneity of slopes tested according to the model:

$$Y_{ij} = u + cow_i + day_j + day_j^2 + (cow * day)_{ij} + (cow * day^2)_{ij} + e_{ij}$$

Where:

Y_{ij} = dependent variables,
u = general mean,
cow = cow effect (class variable),
day = linear effect of day (continuos variable),
day^2 = quadratic effect of day (continuos variable),
(cow*day) and ($cow*day^2$) = interaction terms, and
e = error term (0, s^2).

All means reported are least square means unless otherwise indicated. Changes in sward height before and after grazing with the days of regrowth were described using the UNIVARIATE procedure of SAS (34).

Within each grazing session bite rate was registered every 10 minutes. Linear and quadratic effect of grazing time on bite rate was tested with the GLM procedure of SAS (34) as in a repeated measurement design:

$$Y_{ijk} = \mu + cow_i + day_j + (cow \times day)_{ij} + TL_k + TQ_k + (TL \times day)_{jk} + (TQ \times day)_{jk} + e_{ijk}$$

Where:

Y_{ijk} = bite rate,
μ = general mean,
cow = cow effect (class variable), i = 1 to 4,
day = day effect (class variable), j = 1 to 5,
cow × day = interaction term,
TL = linear effect of grazing time, k = 1 to number of ten minutes units of observation for each individual cow (n),
TQ = quadratic effect of grazing time, k = 1 to n,
(TL × day) and (TQ × day) = interaction terms, and,
e_{ijk} = error term (0, s^2).

To test day and cow effect the interaction (cow × day) was used as the error term.

RESULTS

Weather conditions during the experiment are presented in Table 1. The information was collected from a meteorological station (Maandoverzicht Weergegevens Station Wageningen "De Haarweg") located approximately 300 m from the experimental plot. Except for experimental days 16 and 22, which exhibited higher temperatures and lower relative humidity, weather conditions were relatively stable. However due to rain each day (mean: 2.36 h/d) from experimental day 9 to 13 and symptoms of illness shown by one cow on day 13, data from day 13 were omitted from the analyses.

TABLE 1. Weather conditions during the experimental days.

Day of regrowth	Date	Air Temp. °C	Relative Humidity %	Wind $m\ s^{-1}$	Rain fall mm
6	20-05-96	11.0	59.0	2.5	0.0
9	23-05-96	12.7	83.0	5.9	3.3
13	27-05-96	11.0	86.0	5.1	4.0
16	30-05-96	20.4	59.0	3.0	0.0
22	05-06-96	21.6	51.0	3.4	0.0
30	13-06-96	13.5	61.0	2.5	0.0

Data source: Maandoverzicht Weergegevens Station Wageningen "De Haarweg".

The sward characteristics before grazing are given in Table 2. Sward N content exhibited a significant deviation from linearity. No important changes were evident on grass N content during the first two weeks of regrowth but a significant decline occurred thereafter. A similar trend, but in opposite direction, was observed for sward NDF content. No clear trends were observed for herbage OM content. Sward DM content decreased during the first 16 days, and thereafter increased significantly. Description of available and refused sward mass height is presented in Table 3.

Grazing behavior and rumen pool data after grazing are summarized in Table 4. Neither DMI nor intake rate increased with the age of regrowth. Both GT and eating time exhibited profound discontinuities with age of sward regrowth. A large increase was observed from day 6 to day 9, then a decrease from days 9 to day 16, and then a relatively constant value up to day 30. Nevertheless neither the linear nor the quadratic term were significant. Bite mass tended to increase ($P<0.08$) with period of regrowth, although neither MBR nor adjusted bite rate showed any such effect. Bite rate was not uniform during the grazing session, exhibiting a significant linear decline with time (Figure 2). Solid (wet), total and DM rumen pool sizes after grazing increased linearly with age of regrowth. DM clearance rate declined with the days of regrowth. Milk production before each measurement day exhibited random variations during the experiment (Table 4).

DISCUSSION

Experimental Protocol

We have worked with tethered grazing animals as experimental units in previous experiments (3, 4) as a technique to run controlled grazing experiments. After a short training period the animals become used to the procedure and exhibit a normal behavior. Nevertheless conclusions from this work have to be treated carefully because of some limitations of the experimental protocol. We succeeded in achieving the target herbage allowance per cow during each grazing session, irrespective of the days of regrowth, but sward height and chemical composition were confounded in this experiment. Although we did not expect carry-over effects from one experimental day to another (7) the protocol did not allow us to estimate or eliminate them.

TABLE 2. Plot areas, sward masses and chemical composition of herbage before grazing.

	Days of regrowth						Linear	Q[1]	
	6	9	13	16	22	30	Slope	Slope	SEM
Plot area, cm^2	299	165	123	87	71	57			
Sward Mass, kg DM $hectare^{-1}$	907	1022	1466	1797	2524	4007	129***	NS	248
DM content, g kg fresh $grass^{-1}$	201.5	192.5	166.0	150.7	189.0	199.7	0.86**	0.024**	2.24
OM, g kg DM^{-1}	904.8	902.6	892.7	893.1	906.8	909.4	-1.87	0.05	7.09
N, g kg DM^{-1}	40.4	42.6	43.3	43.5	34.8	29.0	0.85	-0.039**	3.54
NDF, g kg DM^{-1}	445.7	424.9	423.0	444.4	466.9	531.9	-5.9**	0.272***	16.82

***, $P < 0.01$; **, $P < 0.05$; [1]Q = quadratic; [2]NS = non significant

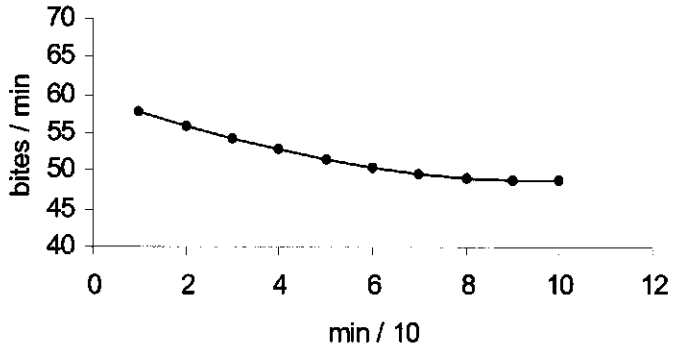

FIGURE 2. Relation between grazing time (X, individual units of ten min) and bite rate (BR).
Model: BR = 59.9 (± 2.1) – 2.25 (± 0.81) X+ 0.11 (± 0.06) X^2, SEM = 9.64.
The negative linear effect of X was significant ($P<0.01$) but the quadratic effect (X^2) not.

Sward Measurements

The sward exhibited a mean daily increase in mass and height of 129 kg DM ha^{-1} and 0.93 cm, respectively, which could be considered as a good growth rate for early spring (24, 35). Herbage CP content in the grass samples cut above 2.5 cm on days 22 and 30 (218 and 181 g kg^{-1} DM) are similar to the figures of 190-200 g CP kg^{-1} DM, provided by CVB (5) as an indication of Dutch swards' CP contents at yields of 1.7 –3 t DM ha^{1}. Both lower (35) and higher (39) values of ryegrass N content have been reported for similar stages of regrowth. Higher values of N, reported by Van Vuuren et al. (39), may have been due to the higher sward cutting height (4-cm) use by these authors. The low N content in the grass samples reported by Steg et al. (35) was due to the weather conditions during their experiment resulting in a rapid maturation of the pasture. The N content of the herbage increased moderately from day 1 to 16 and then declined rapidly (Table 2). Van Vuuren et al. (39) observed the same trend measured over a longer period (week 1 to 8). In our experiment, the stubble height could have influenced the chemical analyses during the first weeks of regrowth, since the experimental plot was mown at 4-cm and the individual experimental samples were cut at 2.5-cm. Nevertheless the significant decline in sward N content after 16 day of regrowth may have been due to an increase in the proportion of stem, or a decrease in N content in leaf and stem fractions or both (33). Herbage NDF content was within the range of values reported by Van Vuuren et al. (39) and Steg et al. (35). As with N, sward NDF content changed little during the first two weeks of regrowth, then increased rapidly, agreeing with the pattern observed by Van Vuuren et al. (39). A slight decrease in NDF content during the first week of regrowth may be a reflection of an increase in the leaf to stem ratio, compensating for the effect of maturation on the fiber content and quality.

The increase in sward height heterogeneity before and after grazing as regrowth period increased is noteworthy because of the influence of this variable on intake rate (22). Although the grazing plots on day 30 were the smallest, cows appeared to concentrate their grazing on shorter denser areas of sward, systematically re-grazing those areas, whilst appearing to avoid areas of taller, sparser sward. Normally heterogeneity in tiller length and density occur at scales smaller that feeding station (22), and the overlapp of areas attempted in new bites with areas previously bitten may have prevented a higher intake rate in the tallest treatments (Table 2). The depth of the horizon grazed during the grazing session increased with the age of regrowth (Table 3), in agreement with previous research (e.g. 10, 21, 40). However, when the sward height removed was expressed as a fraction of the initial sward height (HP, Table 3) non-significant increments were observed after 16 days of regrowth, suggesting that the cows removed a constant portion of the available sward height (23, 40). On the shorter swards (measurement days 6 and 9) a deeper grazing horizon was probably inhibited by the presence of pseudostems (1, 8).

TABLE 3. Sward height data before and after grazing.

Regrowth Days	Sward A,[1], R,[2]	n	Mean cm	Median cm	Min.,[3] cm	Max.,[4] cm	Q25[5] cm	Q75[6] cm	HP[7] %
6	A	131	6.18	6.25	3.0	9.0	5.5	7.0	11.6 b
	R	194	5.46	5.40	3.0	8.5	4.6	6.0	
9	A	189	6.56	6.50	3.5	12.0	5.8	7.5	10.5b
	R	193	5.81	5.70	3.0	9.0	5.0	6.7	
16	A	176	13.0	12.9	8.0	20.4	11.5	14.4	21.7a
	R	173	10.2	10.2	4.8	16.0	8.6	11.5	
22	A	154	19.5	19.2	12.3	28.0	17.1	22.0	27.8a
	R	150	13.8	13.5	7.1	24.0	11.5	15.7	
30	A	154	27.9	28.3	13.5	38.0	25.4	30.9	26.8a
	R	123	20.3	21.0	8.0	32.2	15.8	24.4	

[1]A = sward mass available before grazing
[2]R = sward mass remaining after grazing
[3]Min. =minimum
[4]Max. = maximum
[5]Q25 = quartile 25 %;
[6]Q75 = quartile 75 %
[7]HP = sward height reduction as a percentage of the sward height measured before grazing
[a,b] Within-column means not sharing a common letter differ significantly ($P < 0.01$).

TABLE 4. Effect of the days of regrowth on short-term DMI, intake rate grazing behaviour and rumen pool sizes and kinetics.

	Days of regrowth							
	6	9	16	22	30	SEM	Linear[3]	Q[4]
Milk production, l d^{-1}	26.2	23.7	27.3	29.0	22.5	3.95	NS	
Dry matter intake, kg	2.8	3.3	2.6	3.1	3.5	0.81	NS	NS
Intake rate, kg h^{-1}	2.1	1.2	2.1	2.0	2.2	0.74	NS	NS
Grazing behaviour								
Grazing time (GT), min	89.5	166.7	92.0	94.5	98.7	44.6	NS	NS
Eating time, min	82.7	160.7	82.5	90.0	97.2	43.8	NS	NS
MBR,[1] bites min^{1}	56	51	50	55	50	6.2	NS	NS
ABR,[2] bites min^{1}	58	52	53	57	51	5.5	NS	NS
Bite mass, g $bites^{1}$	0.62	0.40	0.66	0.61	0.72	0.18	NS	NS
Rumen pools size after grazing								
Solid (wet), kg	47.4	48.5	57.2	61.3	67.6	3.24	***	NS
Liquid, kg	7.0	15.0	11.9	12.3	11.0	3.69	NS	NS
Total, kg	54.4	63.5	69.1	73.6	78.6	4.52	***	NS
DM, kg	6.3	6.9	8.8	8.9	10.1	0.74	***	NS
Rumen pool kinetics								
DM clearance rate, % h^{1}	8.5	6.3	6.0	6.2	5.2	1.30	**	NS

[1]MBR = mean bite rate
[2]ABR = adjusted bite rate
[3]Linear = linear effect
[4]Quadratic = quadratic effect
[5]NS = non-significant.
**$P < 0.05$
***$P < 0.01$

Dry Matter Intake

Neither DMI nor intake rate exhibited significant changes with age of regrowth. The discontinuities observed on DMI between days 6 and 16 are noteworthy. As DMI under grazing is the product of grazing time and intake rate (17) results will be discussed in this terms.

Grazing time. In accordance with the results of previous research (e.g., 10, 17) we expected a maximum meal duration on the shortest sward, with a decline in GT as sward height increased and then a plateau after a certain threshold level had been reached. On measurement day 9 the cows did graze longer than on days 16, 22 and 30, agreeing with previous research, where cows have attempted to compensate for reductions in bite mass and intake rate on short swards by increasing grazing time. However on day 6, the cows did not graze longer. Whether the longer grazing time on day 9 was a consequence of a reduced bite mass due to surface moisture or the small bite mass the consequence of a progressively depleted grazing horizon (22) due to a larger grazing session is arguable. A further cause of the observed decline in GT may be the ability of cattle to anticipate the move to the next strip (19) or the presence of a physical limit to grass prehension (1, 8). In our experimental protocol the grazing sessions were followed by a long period of starvation (Figure 1), so that if the cows were able to anticipate the protocol, they should have been strongly motivated to graze. It is more probable that sward structure limited grazing. In short grasses the height of pseudostems has been considered a potential barrier for grazing (1, 8) and may have prevented subsequent grazing after the first grazing horizon had been removed. Even if the grazing horizon were not completely removed, the sward would become shorter and sparser as the grazing session progressed, a sward condition less preferred by cattle (6). A reduction in grazing time has also been reported under strip grazing (25), under controlled grazing conditions, with hand-constructed swards (36) and with continuous grazing (32), when cows have been exposed to short swards. After day 16, GT showed only small variations (Table 4) and the observed values of GT in this period were within the range of reported values for the first grazing bout (3, 32).

Intake rate. Intake rate is the product of bite mass and bite rate (23, 29). Estimated bite masses were the smallest on experimental day 9 and the largest on day 30. The low sward height on experimental day 9 undoubtedly restricted bite mass (10, 17, 21, 29) due to a restriction in bite depth (23), and could not be compensated for by either bite rate (21) nor by density of the grazed horizon (40). However bite mass tended to be heavier on experimental day 6 than on day 9, even though the sward height and mass were less than on day 6. Rook et al. (32) and Soca (unpublished) working with very short swards (approximately 4 cm), found heavier bite masses when the GT of the cows was reduced either intentionally or as consequence of a supplementation with concentrates. Two factors may be related to the observed differences in bite mass between days 6 and 9. Firstly, it has been shown that bite mass declines with residence time in the plot (6) and that grazing may be seen as a depletion process (22, 40). Therefore, if the animal selects the larger potential bite first and decided to stop the grazing session before this grazing horizon has been completely depleted they will achieve a large bite mass and intake rate, although only sustainable for a short period of time. An extension in grazing time under similar sward height as occurred on day 9, will necessarily be at the expense of a reduction in bite mass and intake rate. Secondly, although it was not rain during the grazing session on day 9 (Table 1), it had rained earlier that morning.

Gibb et al. (11) postulated that surface moisture and lubricity of the laminae might increase slippage between the incisor and dental pad resulting in a lighter bite mass. Thus, to compensate for the resulting reduction in bite mass (Table 4), cows may have attempted to extend their grazing session, since bite rate could not be increased sufficiently to maintain intake rate.

Average bite rate was not affected by the days of regrowth (Table 4). However, cows started the grazing bouts with a high bite rate independent of the initial sward conditions, in line with our previous result (4). These initial, high rates were probably the result of the period of starvation (4, 16) and also possibly, induced by a feeling of hunger exacerbated by the rumen emptying procedure prior to grazing. Laca et al. (21) have shown that bite rate does not necessarily increase with bite mass, since the cows are able to overlap biting and chewing jaw movements. Although not directly comparable with the experiment of Laca et al. (21) bite mass in this study (Table 4) was within the range (0.5 to 1.5 g bite^{-1}) in which the overlapping mechanism was effective (21). As we found previously (4), the high bite rates measured during the first minutes of the grazing session were not sustained throughout the meal (Figure 2). The analysis of the effect of grazing time on bite rate showed a significant linear decline ($P<0.01$) during the grazing bout. The lacks of significance of the interaction terms in the model presented in Figure 2 reflect that the slopes were not significantly different between treatments. There was a significant individual effect (cow effect, $P<0.01$), but no day effect, which means that despite a natural individual variation, they were not differences in the starting values between the days of regrowth. As explained by Gibb (13) the observed reduction in bite rate as the grazing session progressed may be due to an increasing number of short intra meal intervals, toward the end of the meal.

DMRP

Total and DM rumen pool size after grazing (Table 4), were far below the observed rumen pools in other studies (e.g., 2, 3, 4, 30). Only on day 30 did the observed DM rumen pool size approach that expected of a full rumen (4). The DMRP size per se is not a good indicator of short-term rumen fill, because high intake rates of fresh grass could lead to a moderate or even low DMRP size, but still occupying a large space. This apparent contradiction may be explained by the low DM content of the rumen pools after grazing (4), since most of the recently ingested particles may not have been properly chewed releasing the intracellular water (3). Other factors than rumen fill (as represented by total or DMRP size) must have been involved in the termination of the grazing session in this study.

CONCLUSIONS

Control of DMI under grazing is a complex process where information coming from the pasture (sward condition) and from the rumen (rumen fill) or both might signal the end of a grazing session. When grazing shorter swards (6.2 and 6.5 cm for 6 and 9 days of regrowth, respectively) the cows modified GT to compensate for the reduced bite masses and intake rates. However, after 16 days of regrowth sward condition per se did no play an important roll controlling GT. For all regrowth periods evaluated, the cows stopped grazing before a

maximal ruminal capacity was reached. Dry matter rumen pool is unlikely to be a good indicator of short-term rumen fill and was hardly related to GT. It must be born in mind that probable a combination of signals rather than a single signal may control the initiation and termination of a meal (9, 26). The relationship "ingestion-digestion" under grazing requires further research.

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6

EFFECT OF DURATION OF REGROWTH OF RYEGRASS (*LOLIUM PERENNE*) SWARDS ON: 2. RUMEN CONTENT WEIGHT, COMPOSITION AND FERMENTATION OF LACTATING DAIRY COWS

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ABSTRACT

The relative importance of rumen content characteristics and fermentation on the control of grazing time during the first grazing session of the day was studied. Four lactating dairy cows were allowed to graze ryegrass (*Lolium Perenne*) swards, which had been allowed 5 different regrowth periods after mowing (6, 9, 16, 22 and 30 d). The cows were allowed to graze until they stopped voluntarily. Before and after grazing the rumen content was evacuated, weighed, sampled and returned to the animals. Samples of rumen liquid were taken immediately before rumen evacuation and approximately 30, 60, 120 and 240 minutes after the grazing session was finished. Rumen pools sizes of OM and NDF measured after grazing increased significantly ($P < 0.05$) with sward days of regrowth, even though the absolute values were low. Volatile fatty acid rumen pools size increased linearly ($P < 0.05$) with age of regrowth. Concentration of VFA followed a significant quadratic trend with a maximum concentration observed at approximately 110 min after cessation of grazing. Nitrogen fractions (N, ammonia) did not follow any significant trend with age of regrowth. In this study, rumen fill (as represented by total, DM or NDF rumen pools size), VFA (either total or major components), ammonia, pH and osmotic pressure as individual variables were not correlated with grazing time or DMI. It would appear that, rather than a single absolute level of any of these variables being responsible, a combination of these signals may control the initiation and termination of individual meals.
(**Key words:** grazing, rumen content, fermentation, rumen fill, dairy)

Abbreviation Key: OP = osmotic pressure, **BG** = before grazing, **AG** = after grazing.

INTRODUCTION

Control of DMI under grazing is a complex process where information coming from the pasture (sward condition) and from the rumen (rumen content and fermentation products) or both may play a major role. (12). If DMI is seen as the sum of individual discrete meals (13), understanding what causes an animal to start and stop eating could lead to a better understanding and prediction of the daily DMI. For lactating dairy cows two main grazing bouts have been observed: one in the morning and the largest in the afternoon (29). Nevertheless, little research has been carried out to understand the mechanism that control short term DMI under grazing (7, 8).

For grass-fed animals, the physical theory of DMI regulation has been widely accepted (11, 13, 25) even though with criticism (e.g., 18). However under grazing conditions there is no clear evidence of physical control of DMI. Behaviour (29) and concentration of fermentative end products (35) have been postulated as controlling DMI in the grazing ruminant. Reduction in DMI due to the infusion of VFA, either into the rumen or the blood stream, has

been extensively studied (e. g., 11, 13, 18, 23). In general, a dose response relationship has been observed between the level of VFA infused and the reduction in DM intake (11, 13), which would suggest effects of VFA over a wide range of concentrations. Nevertheless, doubt remains as to the extent to which the reduction in DMI was due to the quantity VFA infused or to changes in osmotic pressure (17) and or blood insulin levels (17, 23).

This study was undertaken to examine the relative importance of rumen content weight and composition, fermentation end products, and pH and osmotic pressure, on the control of grazing time and short term DMI. Modification on ingestive grazing behavior (bite mass, bite rate and intake rate) and rumen fill (total and DM rumen content size) due to differences in sward days of regrowth were reported elsewhere (5).

MATERIAL AND METHODS

General procedure

The treatments comprised different periods of regrowth. At day 0 (May 13) the whole pasture was cut with a mowing machine (cutting height 4-cm) and the cuttings removed. On days 6, 9, 16, 22 and 30 after cutting (referred to as measurement days) the cows grazed whilst tethered (7) during their first grazing bout of the day. After morning milking, rumen evacuation of the four cows was conducted between 9.00 and 11.00 h a.m. At 11.00 h the four cows were placed in their respective grazing plots and allowed to graze until they stopped voluntarily, as defined previously (5). Immediately after grazing, each cow was removed to the barn and the rumen was evacuated again. After replacement of this second rumen evacuation, the cows were starved till the next morning when a third rumen evacuation was carried out. Immediately before each rumen evacuation and at approximately 30, 60, 120 and 240 min after the grazing session was finished the rumen liquid was sampled for pH, ammonia, VFA and osmotic pressure (**OP**) determinations. A detailed description of the cows, pasture management and determinations is published in the companion paper (5).

Rumen fluid

Samples of rumen fluid (approximately 250 ml) were collected using a 85 cm plastic tube (2.5-cm diameter), closed at the bottom and with about 270 holes (1.5-mm diameter) drilled in the lower 27 cm. This tube was inserted into the rumen through the cannula and positioned in such a way that the bottom of the tube reached the liquid phase in the ventral rumen sac. A flexible tubing (0.5-cm diameter) was placed into this plastic tube and the rumen liquid siphoned into a plastic bottle. The collected rumen fluid was sieved through a sieve with apertures of 0.04 mm^2, and samples were taken for pH, OP, ammonia and VFA determinations. pH was measured immediately after collection (pH electrode type 62, Testo 252, Testo GmbH & Co, Germany). A subsample (5-ml) was mixed with 5 ml of TCA and kept frozen at –20 °C until analysed for ammonia. For analysis, the subsamples were thawed at room temperature, centrifuged for 10 minutes at 2564 g (Sigma 2-15, Laborzentrifugen GmbH, Germany), and the ammonia concentration in the supernatant liquid determined by

transforming the ammonia with phenol and alkaline hypoclorite into indophenol-blue (31). The concentration of indophenol-blue was measured spectrophotometrically at a wavelength of 623 nm (Beckmann DU 64, Soft Pac module Quant II, Beckmann Instruments, Inc, USA). Another subsample (10 ml) of the rumen liquor sample was acidified with 0.5 ml 85% phosphoric acid and kept frozen at –20 °C pending analysis for VFA. For VFA analysis the samples were thawed at room temperature and centrifuged for 10 minutes at 13600 g (IEC Centra-M, International Equipment Company, USA). Subsequently 500 µl of the supernatant liquid were mixed with 200 µl water and 300 µl of an internal standard (iso-caproic acid). One ml of this mixture was injected into a gas-liquid chromatograph (Packard Becker model 419, packed column filled with chromosorb 101, carrier gas N_2 saturated with formic acid, temperature 190 °C). The VFA's analysed were acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acid. The total concentration of VFA in the rumen liquor was calculated as the sum of the individual VFA. Another subsample of the rumen liquid (5-ml) was kept frozen at –20 °C until analysis for OP. After thawing, the samples were centrifuged for 10 minutes at 2564 g and diluted twice with distilled water. OP was determined, by freezing point depression (Halfmikro-Osmometer; Knauer&Co Gmbh, Germany).

Rumen Samples

The rumen evacuation and sampling procedure are reported elsewhere (5). Dry matter content of the rumen samples was determined by freeze drying to constant weight. Freeze dried samples were analysed for ash, N, NDF and Acid Detergent Lignin (ADL). Ash was determined in a muffle furnace at 550 °C, and N by the Kjeldahl method with $CuSO_4$ as catalyst. Neutral detergent fiber was determined according to Van Soest et al. (33) as modified by Goelema et al. (16) and ADL was determined according to the method of Van Soest (34).

Statistical Analysis

Because the treatments were sequentially applied to the same cows over time, first the analysis of a compound symmetric variance structure was tested using the MIXED procedure with cow as a repeated subject (30). As the compound symmetric structure was not significant a general model of heterogeneity of slopes was applied using the GLM procedure of SAS (30).

Rumen pools. Rumen pool sizes before and after grazing were analyzed according to the following model:

$$Y_{ij} = \mu + cow_i + day_j + (cow \times day)_{ij} + e_{ij}$$

Where:

Y_{ij} = dependent variables,
μ = general mean,
cow = cow effect (class variable), i = 1 to 4,

day = day effect (continuos variable), j = 1 to 5,
cow × day = interaction term, and
e_{ij} = error term (0, s^2).

Concentration of fermentative end products. Linear and quadratic effect of sampling time on pH, VFA, ammonia and OP were tested with the GLM procedure of SAS (30) as follows:

$$Y_{ijk} = \mu + cow_i + day_j + (cow \times day)_{ij} + TL_k + TQ_k + (TL \times day)_{jk} + (TC \times day)_{jk} + e_{ijk}$$

Where:
Y_{ijk} = dependent variables,
μ = general mean,
cow = cow effect (class variable), i = 1 to 4,
day = day effect (class variable), j = 1 to 5,
cow × day = interaction term,
TL = linear effect of time of sampling, k = 1 to 5,
TQ = quadratic effect of time of sampling, k = 1 to 5,
(TL × day) and (TC × day) = interaction terms, and,
e_{ijk} = error term (0, s^2).
To test day and cow effect the interaction (cow × day) was used as the error term.

TABLE 1. Significance of the main effects tested (cow, day and their interaction) on rumen pool sizes before and after grazing.

	Evacuation before grazing			Evacuation after grazing		
Variable	Cow	Day	Cow × Day	Cow	Day	Cow × Day
Total rumen pool, kg	**	***	NS	NS	**	NS
DM content, %	NS	**	NS	NS	NS	NS
Particulate matter						
OM rumen pool, kg	NS	***	NS	NS	**	NS
N rumen pool, kg	NS	**	NS	NS	NS	NS
NDF rumen pool, kg	NS	***	NS	NS	**	**
ADF rumen pool, kg	NS	***	NS	NS	**	NS
ADL[1] rumen pool, kg	NS	***	NS	NS	NS	NS
Liquid phase						
Acetic, mol	NS	***	NS	NS	**	NS
Propionic, mol	**	***	NS	NS	**	NS
Butyric, mol	***	***	NS	NS	**	NS
Branched,[2] mol	NS	***	NS	NS	NS	NS
Total VFA, mol	**	***	NS	NS	**	NS
Ammonia, g	***	NS	NS	NS	NS	NS

***$P < 0.01$;
**$P < 0.05$;
[1]ADL = acid detergent lignin, and,
[2]Branched, = iso-butyrrc + isovaleric.

RESULTS

Statistical significance of the effect of cow, days of regrowth and their interaction on rumen pools sizes before (**BG**) and after (**AG**) grazing are shown in Table 1. Least square means, slopes and standard error of the regression analysis are shown in Table 2 and 3. Rumen pools size BG (with exception of ammonia) exhibited a significant linear increment with days of regrowth (Tables 1). Because of the linear trend observed, the rumen pools size BG was included as a covariant in the regression model for rumen pools size AG. The interaction effect between cow and days of regrowth was not significant for any of the measured variables (except NDF) either BG or AG. This means that, despite some differences between cows in initial absolute rumen pool sizes, the linear effect of age of regrowth was the same for all the cows. Total rumen pool size AG increased significantly ($P < 0.05$) with age of regrowth, However DM content did not change significantly (Table 1) although the exhibited trend to increase (Table 2). Neutral detergent fiber and ADF rumen pools increased significantly ($P < 0.05$) with days of regrowths but ADL rumen pool did not. Total and major VFA fractions followed the same trend than NDF and ADF. There was no significant trend in the N fractions, either in the particulate (N rumen pool) or in the liquid phase (ammonia).

TABLE 2. Total (kg), DM content (DM, %), organic matter (OM, kg), nitrogen (N, kg), neutral detergent fibre (NDF, kg), acid detergent fibre (ADF, kg) and acid detergent lignin (ADL, kg) rumen pools size before (BG) and after (AG) grazing.

Rumen Pool		Days of regrowth 6	9	16	22	30	Slope[1]	SEM
Total kg	BG	49.2	51.4	64.7	67.5	73.8	1.06	5.02
	AG	54.4	63.5	69.1	73.6	78.6	0.71	4.60
DM %	BG	10.9	10.3	12.2	11.3	12.0	0.057	1.00
	AG	11.7	10.8	12.6	12.1	12.9	0.036	0.68
OM kg	BG	4.76	4.65	7.13	6.86	8.00	0.143	0.93
	AG	5.60	6.09	7.86	7.91	9.13	0.088	0.61
N kg	BG	0.23	0.20	0.34	0.31	0.31	0.0043	0.06
	AG	0.29	0.30	0.40	0.37	0.36	0.0008	0.04
NDF kg	BG	2.05	2.29	3.34	3.31	4.16	0.085	0.38
	AG	2.22	2.67	3.44	3.49	4.69	0.059	0.30
ADF kg	BG	1.22	1.36	2.02	1.91	2.38	0.047	0.24
	AG	1.37	1.67	2.10	2.10	2.69	0.028	0.19
ADL Kg	BG	0.25	0.25	0.40	0.40	0.47	0.0098	0.05
	AG	0.28	0.29	0.42	0.42	0.49	0.0005	0.024

[1]Slope = slope of linear effect of days of regrowth.

TABLE 3. VFA and ammonia rumen pool sizes before (BG) and after (AG) the grazing session.

Rumen Pools		Days of regrowth 6	9	16	22	30	Slope,[1]	SEM
Acetic mol	BG	2.64	1.92	2.61	2.88	3.76	0.057	0.572
	AG	2.70	2.75	2.91	3.28	4.20	0.042	0.502
Propionic mol	BG	0.87	0.81	1.11	1.11	1.19	0.015	0.174
	AG	0.88	1.02	1.12	1.17	1.35	0.019	0.172
Butyric mol	BG	0.59	0.51	0.61	0.60	0.78	0.0086	0.102
	AG	0.53	0.65	0.63	0.67	0.98	0.0133	0.146
Branched,[2] mol	BG	0.092	0.065	0.123	0.124	0.156	0.003	0.024
	AG	0.102	0.101	0.115	0.126	0.145	0.0016	0.023
Total VFA mol	BG	4.27	3.43	4.60	4.85	5.99	0.085	0.794
	AG	4.25	4.54	4.90	5.40	6.93	0.091	0.781
Ammonia g	BG	5.14	5.43	9.96	6.65	7.72	0.099	2.347
	AG	9.89	11.5	9.87	8.70	12.1	0.041	3.343

[1]Slope = slope of linear effect of days of regrowth, and,
[2]Branched, = iso-butyric + isovaleric.

In Table 4, the time course of the fermentative end products, pH and OP are shown. There was a significant cow effect for all measured variables (except OP) reflecting high individual variability. The inclusion of a quadratic term was highly significant ($P < 0.01$) for all the fermentative end-product concentrations and OP, indicating the presence of a maximum or minimum within the 4-h period of sampling. Except for rumen ammonia concentration, there was no significant interactive effect of day and sampling time, either linear or quadratic, on the concentration of fermentation products. Fermentative end products concentrations, pH and OP observed AG and predicted by the statistical model are shown in Figure 1.

DISCUSSION

Although the experimental protocol was designed to achieve relatively homogeneous rumen status BG, rumen pool sizes BG did exhibit a significant linear increase with the days of regrowth (Tables 2 and 3). Between measurements days the cows grazed an adjacent plot with a non-limiting availability of grass and in addition we expected low grazing activity at night (7, 29). Nevertheless the temporal pattern of grazing might be altered for weather conditions mainly high temperatures and raining during the day. On rainy days lower grazing activity and DM intake has been observed (11). Measurement days 6 and 9 were both preceded of rainy days while the other experimental days were not and this may have induced a different DMI the days before the determinations took place. Since rumen pool before grazing as such would affect grazing time and DMI (7, 8) conclusion from this work have to be taken carefully because the limitation in the experimental protocol.

TABLE 4. Significance of different effects on VFA, ammonia, pH and osmotic pressure (OP) in rumen liquor after grazing.

Variable	Cow	Day	TL,[1]	TQ,[2]	Day × TL	Day × TQ	Intercept	Slope L,[3]	Slope Q,[4]	SEM
Fermentation products										
Acetic, mmol l^{-1}	***	NS	NS	***	NS	NS	51.6	0.079	-0.00041	6.90
Propionic, mmol l^{-1}	NS	NS	***	***	NS	NS	17.5	0.055	-0.00023	2.75
Butyric, mmol l^{-1}	NS	NS	***	***	NS	NS	10.7	0.037	-0.00015	1.76
Branched,[5] mmol l^{-1}	NS	NS	***	***	NS	NS	1.69	0.011	-0.00003	0.34
Total VFA, mmol l^{-1}	**	NS	**	***	NS	NS	83.3	0.194	-0.00088	11.9
Ammonia, g l^{-1}	NS	NS	***	***	***	***	160.3	0.772	-0.00348	28.6
Rumen environment										
PH	***	NS	NS	NS	NS	NS	6.5	-0.0004	0.000004	0.19
OP, mosmol ml^{-1}	NS	NS	NS	**	NS	NS	307	0.0307	-0.00069	20.8

[1]TL, = linear effect of time of sampling,
[2]TQ, = quadratic effect of time of sampling,
[3]Slope L, = slope of linear effect,
[4]Slope Q = slope of quadratic effect, and,
[5]Branched, = iso-butyric + isovaleric.

Total and OM rumen pool sizes AG (Table 2), were far below the observed rumen pools sizes in other studies (e.g., 3, 4, 7, 28). Bosch et al. (4) summarized values of total rumen pool from 18 to 25 g per kg body weight for cows eating silage (lower values) and hay (higher values) which contrasts with our observations (10 to 14.7 g per kg body weight). Lower OM rumen pool size AG during the first two measurement days might have been related either to the higher rumen DM clearance rate of the first measurement days (5) or to the low DMI imposed by the sward height of the available pasture. Changes in sward chemical composition may have also play a role in the observed rumen pools after grazing. The analysis of the grass samples fermentation characteristics using the gas production technique (6), showed a linear decline both in gas production and in the extent of fermentation during the regrowth period.

Rumen DM percentage of rumen content AG was low and tended to grow with days of regrowth although non significantly. Low DM rumen contents in cows fed with fresh ryegrass (around 10 %) has been reported (7, 38) and might reflect a low chew efficiency during grazing (7). Analysis of rumen particle size distribution after 1 h of grazing on fasted cows showed that more than 75 % of the ingested forage was over 1.25 mm (8) which is greater than values previously reported for cattle fed with fresh grasses and legumes (38, 39). An increase in rumen DM content associated with a greater total rumen pool as we did observe (Table 2) may have consequences for the trapping of small particles (1) and eventually might prevent high values of passage at high levels of intake (28).

Rumen NDF pools AG on measurements days 6 and 9 were comparable with NDF pools observed in grazing dairy cows after overnight starvation (7) but lower that NDF rumen pools after 1 h of grazing. Van Vuuren et al (36) observed low NDF rumen pools (2.27 kg) in dairy cow fed highly fertilized fresh ryegrass, probably associated with a low DM content of the grass. Low NDF rumen pools has been also observed in dairy cows fed fresh ryegrass and supplemented with 7 kg of a high starch concentrate (35). From day 16 onwards NDF rumen pools AG were greater than on days 6 and 9 (Table 2), but still within the lower range of NDF rumen pools reported elsewhere (e.g., 4, 8, 9, 14, 19, 21, 22, 28). The highest NDF rumen pool measure on measurement day 30, was below the threshold level of 1.1-1.2 % of body weight described by Mertens (25) as the average NDF holding capacity of dairy cows on a daily basis. It seems clear that NDF rumen pool as a individual entity was not the main signal received by the cows to stop the grazing session.

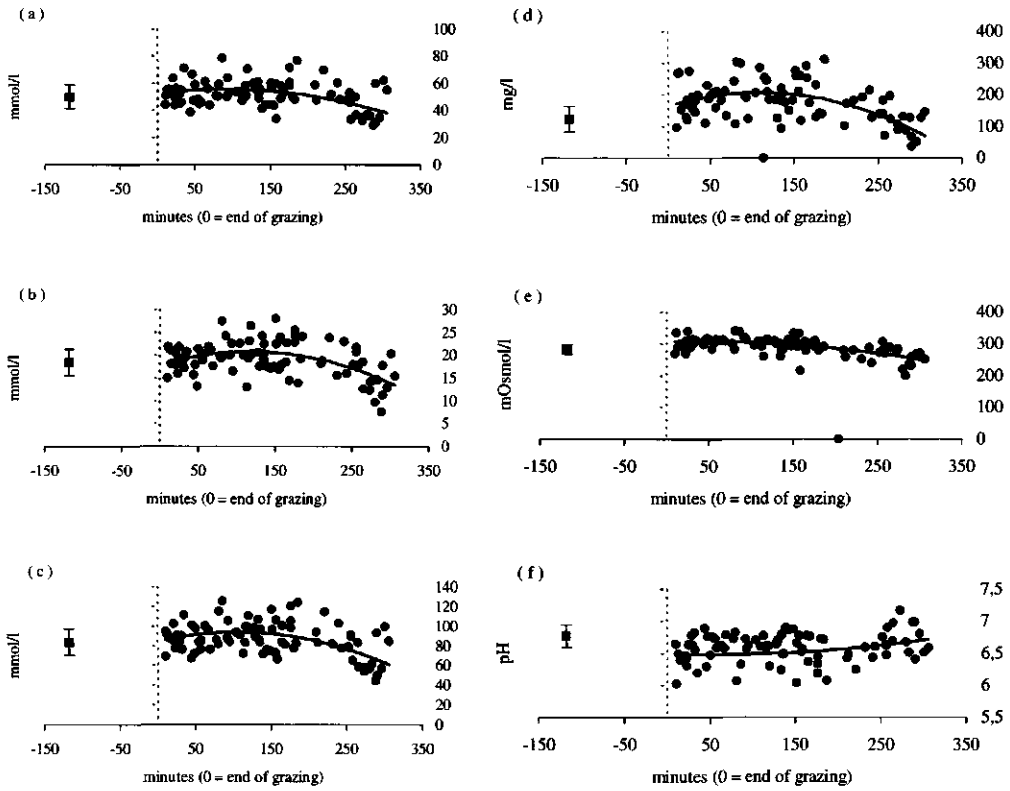
VFA rumen pool sizes (either total or major VFA) increased linearly with the days of regrowth (Table 3). DMI reduction in response to the infusion VFA, either in the rumen or in the blood stream, has been extensively studied (e. g., 11, 13, 17, 18, 23). In general a dose response relationship has been observed between the level of VFA infused and reduction in DM intake (11, 13) which suggest that slight increases on VFA from the basal levels should start to reduce DMI. In this experiment, VFA rumen pools AG on days 6 and 9 were similar to the rumen pools observed after 1.75 h of grazing following overnight starvation (7). At the other extreme, VFA rumen pool sizes AG measured on day 30 were similar to the maximum rumen pool sizes (approximately 7 mols) observed immediately after the first morning grazing session (7). Nevertheless, the observed VFA rumen pool sizes AG in this experiment were below the range where they would be expected to cause DMI depression (2, 24).

The change in VFA concentration AG, followed a significant ($P < 0.01$) quadratic trend with time (Table 4; Figure 1). It means that if the animals were able to sense rumen VFA

concentration and/or VFA rumen pool sizes (13, 18) they do interrupt the grazing session before this maximum level had been reached (23). Maximum rumen liquid FVA concentrations (either total or major components) were observed at approximately 110 minutes after grazing. Nevertheless the maximum VFA concentration observed was lower than values reported for grazing cattle (27, 37). The lack of a significant interaction between experimental day and time of sampling (either linear or quadratic) indicates a degree of stability between experimental days in the observed pattern.

Ammonia rumen pool sizes were not affected by age of regrowth (Table 3). Ammonia rumen pool size AG was better correlated ($r = 0.65$, $P < 0.01$) with DMI than VFA rumen pool ($r = 0.34$, NS). Chilibroste (unpublished) studying the fermentation pattern during the first grazing session a.m. of lactating dairy cows found that ammonia concentration in the rumen liquor increased linearly with time while VFA concentration exhibited a plateau during approximately one hour of grazing and then sharply increased. In that study, the samples of rumen liquid were taken at 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 min after the grazing had started. Irrespective of the mechanism leading to these differences, in terms of satiety signals at the level of the rumen, these results suggest that perhaps ammonia is more important than VFA level in regulating meal under grazing. Although the strong association observed between ammonia concentration on silage and DM intake (15) not direct effects of ammonia per se at rumen level has been demonstrated (32). High levels of ammonia in the rumen have been tolerated by animals offered good quality silage, without any reduction in DMI (32). In cattle grazing leafy young temperate pastures high peaks of ammonia (300 to 500 mg/l) after grazing are non unusual (27, 37). Ammonia detoxification by the liver and or a shortage in the supply of energy required for this process has been suggested to be involved in the metabolic control of DM intake (20). Short term unbalances in the supply of ammonia and VFA as our results suggested should be considered in further research (20). Like the rumen ammonia pool, rumen liquid ammonia concentration AG did not follow a clear trend (Table 4; Figure 1) with days of regrowth. The branched VFA (isobutyric + isovaleric) did not change with the days of regrowth, although the chemical composition of the grass, particularly N content, did change significantly with grass maturity (5). This trend might reflect changes in N utilisation (6), a better synchrony of carbohydrates and nitrogen availability in rumen (10, 36) or simply that rumen microbes that require branched VFA can only take up branched VFA if concentration is above this level.

Following Grovum (18) further experimental evidences as to the role of OP in the control of food intake has been forthcoming (13, 17), and in some instances striking (24). Grovum (17) has shown dose-response reductions in DMI with the addition of NaCl in the rumen of sheep. Like in our experiment a high variability between animals on OP values has been observed (17). The time course AG of the rumen liquid OP exhibited a significant ($p<0.05$) quadratic term (Table 4; Figure 1) with the maximum value been observed around 22 minutes after the end of the gazing session. In this experiment osmotic pressure values AG were within a normal range (12) and far below the level of range 450 to 550 mosmol l^{-1} OP at which DMI either ceased (26) or was severely depressed (17) in sheep.



a = R^2 = 0.75; SEM = 6.90, b = R^2 = 0.72; SEM = 2.75, c = R^2 = 0.72; SEM = 11.90,
d = R^2 = 0.86; SEM = 28.6, e = R^2 = 0.64; SEM = 20.8 and f = R^2 = 0.81; SEM = 0.17.

FIGURE 1. Fermentative end-product concentration (a, acetic; b, propionic; c, total VFA, d, ammonia) and rumen environmental variables (e, osmotic pressure; f, pH) before (filled square) and after grazing (dots, observed values; lines, predicted values from model Table 4).

CONCLUSION

Rumen fill (as represented by total, DM or NDF rumen pool sizes), VFA (either total or individual components), ammonia, pH and OP when taken in isolation are unlikely to be the responsible for the observed differences in DMI and GT in this experiment. During the first measurements days behavioral restrictions imposed by the sward characteristics appeared to be important in terminating the grazing session. From day 16 onwards, is more probable that a combination of signals generated either at rumen (13, 18) or at metabolic level (20) or both might have been responsible for the observed DMI and GT.

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DESIGN AND EVALUATION OF A NON-STEADY STATE RUMEN MODEL

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ABSTRACT

A dynamic simulation model of digestion and absorption of nutrients (Dijkstra et al., 1996) was modified and evaluated under non-steady state conditions. The results of detailed grazing experiments that concern allowed grazing times (experiment 1), combinations of rumen fill and starvation length before grazing (experiment 2) and contrasting sward masses and heights (experiment 3) as main treatments were used as reference values. The model was modified to run under a discontinuous feed input of ryegrass. Neutral detergent fibre (NDF) and nitrogen (N) rumen pool were predicted with a relatively low root mean square prediction error (MSPE) of the observed means (12 %) for experiments 1 and 2 but higher values (18.1 %) were observed for experiment 3. The root MSPE was significantly inflated by the long period of starvation (length of starvation period up to 20.5 hours) that followed grazing in the three experiments. Organic matter (OM) rumen pool was poorer predicted (root MSPE of 16 %) than NDF and N rumen pools and requires further validation. Volatile fatty acids (VFA) rumen pool and VFA concentration were predicted with a root MSPE of the observed mean of 32 to 33 % which was close to the random variation observed in the experiments. Ammonia rumen pool was poorly predicted and the way ammonia is represented in the model must be modified to predict ammonia production and absorption under non-steady state conditions. It was concluded that the model can be used under discontinuous feeding regimens to predict ruminal digestion and absorption of nutrients (except ammonia) on grazing lactating dairy cows, although feeding regimens that would involve large periods of starvation must be avoided.

Key Words: Dairy Cows, Rumen Model, Non-Steady State, Grazing, Digestion,

INTRODUCTION

Grazing systems rely on the direct utilisation of the produced grass and this is a main issue in terms of productivity and profitability of these systems on market-oriented countries (Clark and Jones, 1995). The problem of grass production and utilisation may be seen from a whole paddock, animal or plant morphology perspective (Parsons and Chapman, 1998). To assess animal productivity from an animal perspective, dry matter intake (DMI) is the dominant process (Forbes, 1995). In forage fed animals the processes that happen in the rumen play a determinant role in the amount and type of nutrients absorbed (Tamminga and Van Vuuren, 1996) and in DMI control (Grovum, 1995). Therefore the subdivision of productivity in intake, digestion and utilisation of nutrients is used to elucidate and clarify possible underlying mechanisms since the high interrelation between these processes is well known.

The progress made in the representation and quantification of the rumen fermentation process in models simulating whole rumen function has been significant, although important gaps in knowledge and representation still remain (e.g. Bannink and De Visser, 1997; Dijkstra, 1994). An extensive evaluation of whole rumen function models has recently been made (Dijkstra and France, 1996) and a number of issues that require further research were addressed. The

need for models able to represent discontinuous feeding regimes, associated with rumen pools varying in size with time, was highlighted (Dijkstra and France, 1996).

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

MATERIAL AND METHODS

A dynamic mechanistic model constructed by Dijkstra et al., (1996) to predict digestion and absorption of nutrients in cattle fed sugarcane based diets in steady state conditions was modified to simulate a discontinuous feeding regimen of ryegrass. Three grazing experiments (Chilibroste et al., 1997; 1998a; 1999 a; b) were used for the evaluation of the modified model. The experiments offered a large data set (n=104 individual observations) obtained within the same research program with lactating dairy cows grazing ryegrass. Most of the inputs required by the model were measured during the experiments. Otherwise they were derived as explained in the input section.

Description of the model

The rumen model comprises 11 state variables representing four carbohydrate fractions, four nitrogen (**N**) fractions, two fatty acid fractions and one microbial rumen pool. The four carbohydrate fractions included undegradable neutral detergent fibre (**NDF**), degradable NDF, insoluble starch, and soluble starch and sugars. The four N fractions included undegradable protein, insoluble but degradable protein, soluble protein, and ammonia. The two fatty acid fractions included long chain fatty acids and volatile fatty acids (**VFA**). All pools were expressed in grams except VFA pools that were in moles. The rate of change of each pool with time was described with a single differential equation integrating inflow, outflow, synthesis and utilisation of each fraction at each time. The flux equations were described by Michaelis-Menten and mass-action forms. For numerical integration a fourth-order Runge-Kutta method was used and run for a number of days to achieve steady state solutions. Details about model parameterisation and all the flux equations that constitute the model have been described in detail in Dijkstra et al. (1996).

Description of the experimental protocols

Detailed descriptions of the experimental protocols have been published elsewhere (Chilibroste et al., 1997; 1998a; 1999 a; b). The general procedure for an experimental day is shown in Figure 1. After morning milking, rumen evacuation of the cows was conducted. After the rumen evacuation the cows were placed in their respective grazing plots and allowed to graze until the allowed grazing time was finished (experiment 1) or they stopped

voluntarily (experiments 2 and 3). Immediately after grazing, each cow was moved to the barn and the rumen was evacuated again. After replacement of this second rumen evacuation, the cows were starved till the evening (experiment 1 and 2) or to the next morning (experiment 3) when a third rumen evacuation was carried out (Figure 1).

Model modifications

To be able to run the model under non-steady state conditions a series of modifications were introduced.

Time. The original model was developed to run with hour as unit of time. Since the grazing observations were done every ten-min interval, the unit of time in the model is minute. The rumen pool sizes measured by evacuation before the grazing session (evacuation 1, Figure 1) were used as initial values at time 0 minutes and the model was run up to and including the time at the end of starvation (evacuation 3, Figure 1). All the time dependent parameters (production, absorption, synthesis, utilisation and fractional degradation or passage rates) were converted to operate on a minute basis.

Dry matter intake. Instead of assuming a steady state with a constant input of nutrients like in the original model (Dijkstra et al., 1996), a discontinuous feed input was programmed. In the grazing experiments DMI was estimated from changes in DM rumen pools before and after grazing (Figure 1) and clearance of DM during the grazing sessions (Chilibroste et al., 1997). Besides, grazing time was accounted as the time elapsed from the moment the cows were placed on the experimental plots until they were removed. During grazing, bite rate was recorded by the same observer at intervals of ten minutes for each cow. The total number of bites was calculated as the product of grazing time and mean bite rate. Average bite mass was estimated as DMI divided by the total number of bites. In the model DMI at each integration step was calculated as the product of the observed bite rate times the estimated bite mass for each individual cow. For detailed methodological protocols of grazing observations see Chilibroste et al. (1997; 1999a).

Rumen pools. Three new rumen pools were calculated from the rumen pools originally conceived in the model. Organic matter (**OM**) rumen pool was calculated as the sum of the crude protein (CP, NAN x 6.25), carbohydrates and long chain fatty acids microbial and non-microbial rumen pools. DM rumen pool was calculated as OM divided by the ash fraction in the DM (114.8 ± 11.7 g kg DM^{-1}; n=52). Additionally a N rumen pool was calculated by summation of the rumen pools of N arising from the feed (undegradable, degradable and soluble N), N in the ammonia pool and N in the microbial pool. It was assumed that the crude protein (CP) content of the microbial biomass was 650 g/kg DM (Dijkstra et al., 1996).

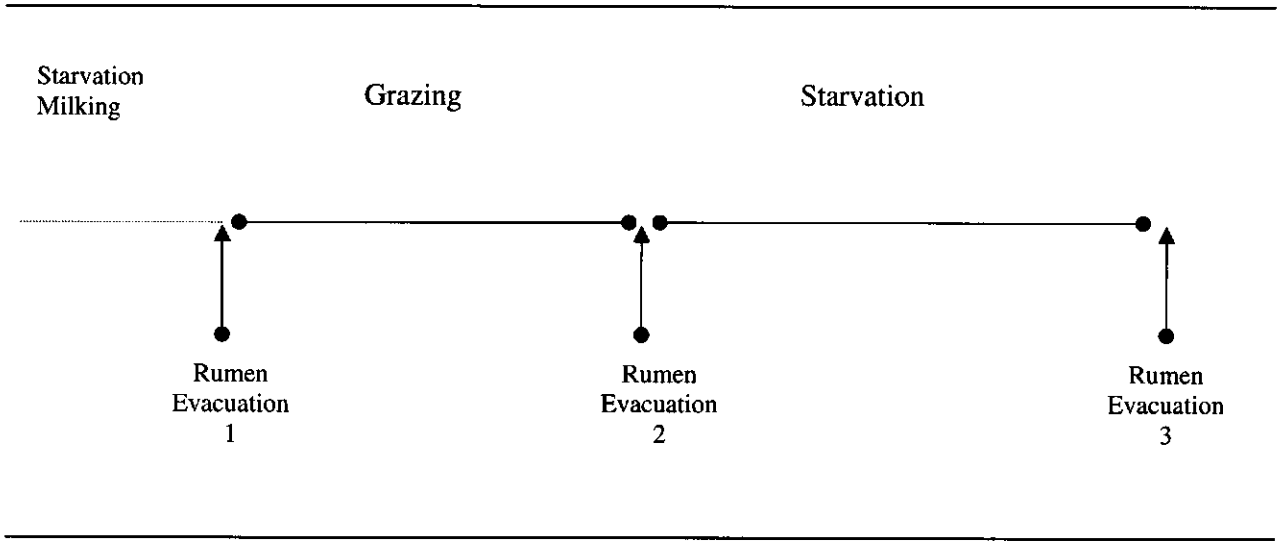


Figure1. Diagrammatic representation of the experimental protocol followed during the experiments.

Rumen volume. To simulate under non-steady state conditions, rumen volume (V) has to be considered as non-steady state variable instead of a constant dietary specific variable as it was represented in the original model. Large variability has been observed in V when low DM content grasses and legumes are fed to cattle (Waghorn, 1986; Chilibroste et al., 1997; 1999a). A non-linear relationship between DM rumen pool size (DMRP; kg) and DM percentage of rumen contents (DMC; %) was derived from the data set (Figure 2) and used to estimate rumen V from DM rumen pool at each model iteration. This yielded the equation:

$$DMC = 12.05\ (\pm 0.19) \times e^{-0.32(\pm 0.17) \times DMRP} \qquad RSE = 1.24.$$

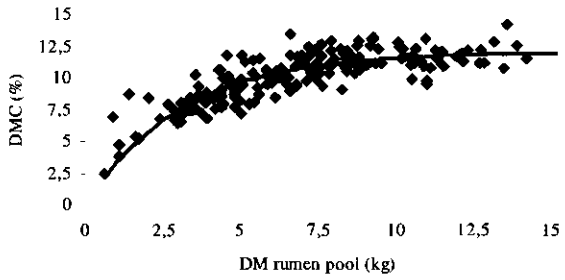


Figure 2. Observed and predicted DM content (DMC, %) in the DM rumen pool (DMRP, kg) for experiments 1, 2 and 3. Symbols denote observed values and the solid line represents the predicted values.

Microbial population. In the original model it was assumed that protozoal and bacterial biomass in the rumen constitute 40 and 60 % of the total microbial biomass in sugarcane fed dairy cattle. These figures were changed to 20 and 80 % (protozoa and bacteria respectively) to represent the situation in cows fed cell wall rich ryegrass diets (Hungate, 1966). Other assumptions about the microbial population, like the proportion of bacteria attached (75%) and non-attached (25%) to the solid fraction and the fractional output rates of protozoa and bacteria, were not modified..

Model input

Feeds. In Table 1 the feed inputs required by the model are summarised for each experiment. Experiment 3 comprised different days of regrowth as treatments and three groups of input values were used. One set of input values was used for the first 16 days of regrowth where little variation on sward chemical composition was observed, and two more sets of input values for measurement days 22 and 30 where changes in sward chemical composition were evident (Chilibroste et al., 1999a).

Table 1. Crude protein (CP), undegradable (PU), degradable (PD) and soluble (PS) CP, neutral detergent fibre (NDF), degradable NDF (NDF Deg.) and water-soluble carbohydrates (CHO Sol) in the grass eaten by the cows.

Fraction ($g\ kg\ DM^{-1}$)	Experiment 1	Experiment 2	Experiment 3 days 6-16	Experiment 3 day 22	Experiment 3 day 30
CP	131.1	262.5	262.0	225.0	181.2
PU	8.5	17.1	17.1	21.7	11.8
PD	76.7	153.5	153.2	127.2	106.0
PS	45.9	91.9	91.7	76.1	63.4
NDF	473.5	511.1	430.1	466.9	531.9
NDF Deg.	378.8	409.2	344.2	373.5	425.5
CHO Sol.	242.2	100.2	150.2	150.0	150.0

The values reported in Table 1 were derived from the grass chemical composition (Chilibroste et al., 1997; 1999a). A constant chemical composition was assumed for each individual bite. The compositional characteristics (U, D and S) of feed carbohydrates and CP were derived from Van Vuuren et al., (1992; 1993) for experiment 1 and 2 and from Chilibroste et al., (1998b) for experiment 3. It was assumed that ammonia, VFA and starch contents of the grass were 0. Also a constant lipid content of 60 g kg DM^{-1} in the grass was assumed. Passage rates of the solid and liquid fractions and degradation rates of degradable NDF and insoluble, degradable CP were assumed to be the same in the three experiments and set at 3.5, 8.0, 4.0, and 8 % h^{-1} (0.058, 0.133, 0.066 and 0.133 % min^{-1}) respectively. This values were derived from Van Vuuren et al. (1992) and from our own nylon bags incubations (Chilibroste et al., unpublished).

Rumen Initial conditions. Initial rumen pools were the rumen pools determined in the evacuation before grazing (evacuation 1, Figure 1). For the three experiments N was assumed to be 10 % soluble, 40 % insoluble but potentially degradable and 50 % non-degradable. Initial NDF rumen pool was estimated to be 50 % non-degradable in experiments 1 and 2 and 40 % in experiment 3. These figures were derived from a simple simulation exercise using NDF and CP rumen pool observed before starvation (Chilibroste et al., 1998a; 1999b) as initial values and with a ratio of degradable to undegradable material as reported by Van Vuuren et al. (1992). The rumen pools were simulated to be exposed to passage (undegradable fraction) and to passage plus degradation (degradable fraction) for the period that the cows were starved. At the end of the simulation period the ratio between degradable and non-degradable fractions were recalculated. The OM residues after 144 h of incubation in vitro (Chilibroste et al., 1999c), after correction for microbial contamination, also gave an indication of degradability of the NDF rumen pools, since NDF made the major contribution to the OM rumen pools (Chilibroste et al., 1998a; 1999a). Both approaches yielded similar figures. Initial lipid rumen pool was set to 60 g kg DM^{-1} and soluble carbohydrates to 0.6 g l^{-1} (Dijkstra, personal communication).

Comparison between simulated and experimental values.

The observed values of OM, NDF, N, VFA and ammonia rumen pools in evacuations after grazing and after starvation (evacuations 2 and 3, Figure 1) were compared with the values

predicted by the model. An assessment of the error of predicted relative to observed values was made by calculation of the mean square prediction error (MSPE):

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

where i = 1, 2, ...n; n is the number of experimental observations and O_i and P_i are the observed and predicted values. The MSPE was decomposed into error due to the overall bias of prediction, error due to deviation of the regression slope from unity, and error due to the disturbance (random variation) (Bibby and Toutenburg, 1977).

RESULTS AND DISCUSION

In Figure 3 the simulated OM, NDF and N rumen pools are plotted against the observed values. The predicted OM pool tended to be lower than the observed pool (root MSPE of 16% of observed mean and overall bias and deviation of the regression slope from unity contributing 33% and 23 % respectively, towards the MSPE). The root MSPE for the NDF rumen pool was also 16% of the observed mean, but without clear bias (80% of MSPE was attributed to the random disturbance proportion). The rumen N pool was predicted well, with a root MSPE of 12% of the observed mean and 80% of MSPE attributed by random disturbance proportion. Because N and NDF pools were fitted well without obvious bias, the poorer fit of OM pool size must result from non-protein, non-NDF organic matter in the rumen, i.e. soluble sugars and crude fat. Since systematic deviations between observed and predicted pools may exist within experiments that will not be apparent in the combined analysis, the root MSPE and the contribution of random variation towards MSPE within experiments are presented in Table 2. For all three pools, the root MSPE of the observed mean was larger in experiment 3 than in experiment 1 and 2.

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

Either an underestimation of OM intake or an overestimation of OM clearance from the rumen might explain a predicted OM rumen pool lower than the observed value. Although discontinuous OM intake was an input during the simulation a constant chemical composition during the grazing session was assumed (Table 1).

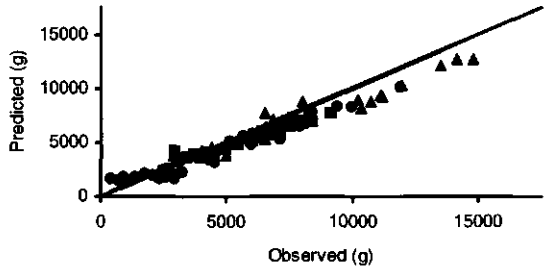
Table 2. Root mean square prediction error (MSPEr, % of observed mean) of organic matter (OM), neutral detergent fibre (NDF) and nitrogen (N) rumen pools and the contribution of random variation towards the MSPE (Random, % of MSPEr) for experiments 1, 2 and 3.

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

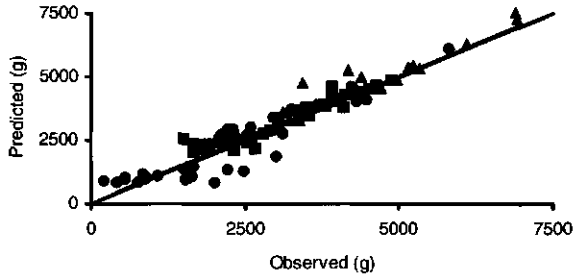
Intake in the experiments was estimated from changes in rumen pool sizes before and after grazing and clearance of DM while grazing. Estimations of DM intake with this approach showed lower variability than when estimated from sward characteristics (Chilibroste et al., 1997). However estimation of selectivity was poor with a high residual variability due to the low sward utilisation in the experiments (Meijs, 1981) and it is likely to be the mean source of error in terms of feed inputs to the model. Clearance out of the rumen is the results of two simultaneous processes: degradation and passage, with the last exerting major effects on model prediction (Dijkstra and France, 1996). In our experiments we did use clearance rate (k_{cl}) of acid-detergent lignin (ADL) as an indicator of solid passage rate, assuming no degradation of ADL occurs. The observed k_{cl} values of ADL (3.9±1.7 % h^{-1}) were within the ample range of passage rates estimated by Owens and Goetsch (1986) and Sauvant et al. (1995) for forages and higher than the value used in the model (3.5 % h^{-1}). However, Van Vuuren et al. (1992; 1993) working with fresh ryegrass reported lower values of passage rate when it was estimated using lignin as a internal marker (2.4 to 3.5 % h^{-1}). Mambrini and Peyraud (1994) also reported lower values of passage for fresh ryegrass labelled with rare earth metals (2 to 2.2 % h^{-1}). Tamminga et al. (1989) pointed out the high variability and low reliability of the use of lignin fraction as internal marker to estimate passage rate.

To investigate if the lower prediction capacity of the model for experiment 3 was associated to the large period of starvation after grazing (average > 20 h) the root MSPE was decomposed by evacuation. The root MSPE of the observed means were 14.8, 11.7 and 6.3 % (evacuation 2) and 35.7, 43.9 and 57.2 % (evacuation 3) for OM, NDF and N, respectively, which indicates that the observations shortly after grazing were predicted more accurately by the model than the observations after a long period of starvation. The decomposition of MSPE by evacuation in experiment 1 and 2 also revealed a higher root MSPE of the observed mean after starvation (average length of starvation of 7.6 h) than after grazing (pooled mean root MSPE for OM, NDF and N was 8.1 and 17.7 % for evacuation 2 and 3 respectively). Important changes in rumen pool sizes and characteristics with starvation time have been reported (Chilibroste et al., 1998a; 1999b) and it is highly probable that a number of the model assumptions (e.g. constant fractional rates for production, utilisation and absorption of nutrients or for fractional passage rates of solids and liquids) does not apply for these rumen conditions. Therefore it is likely that that the model prediction accuracy decays with starvation length or distance from steady state conditions.

a) Organic matter rumen pool



b) Neutral detergent fibre rumen pool



c) Nitrogen rumen pool

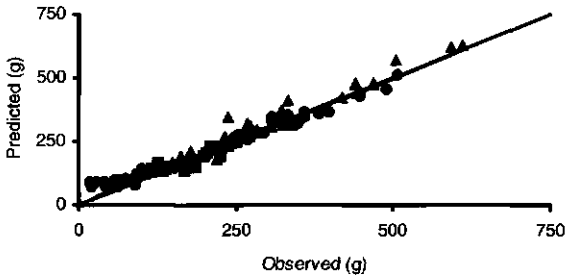
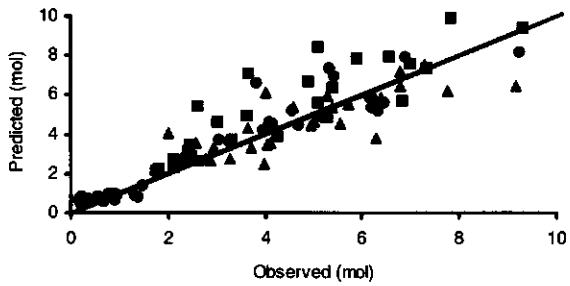


Figure 3. Comparison of observed and predicted values. Observations are: ■, experiment 1; ▲, experiment 2; ●, experiment 3.

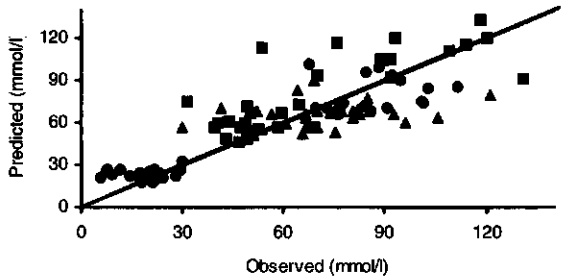
For instance in sheep Aitchison et al (1986) observed that in the first 5 hours after a meal between 50 and 67% of the intake of indigestible NDF (INDF) was lost from the rumen, whereas during that period only between 24 and 52% of NDF intake disappeared. A second increase in the rate of removal was observed between 15 and 24 hours after feeding. Similar observations were made in steers by Thiago et al. (1992). Okine and Mathison (1991) observed differences in duration and amplitude of reticular contractions during eating, ruminating and resting, possibly affecting the outflow of material from the rumen during and after feeding. The functional density of particles varies with incubation time and degradation rate (Nocek and Kohn, 1987) and the escape of particles from the rumen depend on its functional density (Kaske and Engelhardt, 1990). Hence, as rumen particulate OM is being fermented by microbes after a meal, its specific gravity and odds of escape from the rumen may increase or decrease, implying that a fixed passage rate may be incorrect. During fermentation, pH of the rumen fluid may drop due to formation of VFA and a pH below 6.3 may impair activities of fibrolytic bacteria, resulting in decreased rates of degradation of NDF soon after a meal when pH is lowest (Erdman 1988). Also, absorption rates of VFA and ammonia depend on pH of rumen fluid. For example, Dijkstra et al. (1993) observed that VFA absorption rates decreased as pH increased in the range of 4.5 to 7.2. Hence there is ample evidence that fractional rate of passage, degradation and absorption may vary considerably as rumen conditions change.

Simulated and observed values of rumen fluid concentration and pool size of VFA and ammonia are plotted in Figure 4. The VFA rumen pool (Figure 4a) was predicted with a root MSPE of 33 % with a large proportion of the variability explained by random variation (97 %). The VFA concentration in the rumen liquid (Figure 4, plot b) exhibited the same trend (32 and 98 % for root MSPE of observed mean and random variation, respectively). Since VFA production has a major role in energy supply to the ruminant and hence in milk and meat production and product composition (Sutton, 1985), it has received special attention in modelling efforts (e.g. Dijkstra et al., 1992). Nevertheless accuracy of prediction of total VFA, but particularly VFA molar proportions (acetic, propionic and butyric) of existing models remains low (Bannink et al., 1997; Dijkstra, 1994) and a series of suggestions has been done to improve prediction capability (Dijkstra and France, 1996). Regarding the model evaluation in the present experiment, it must be born in mind that high variation coefficients were observed in the experiments (around 30 %) for all the fractions measured in the rumen liquid (Chilibroste et al., 1998a; 1999b). Therefore model prediction accuracy for VFA rumen pools and concentration should not be neglected. In contrast to VFA predictions, the root MSPE of the observed mean for ammonia rumen pool (79 %) and concentration (69 %) were larger. The large error in estimation of ammonia rumen pools and concentrations suggest that the model representation of the ammonia rumen pool dynamics was not adequate (see below).

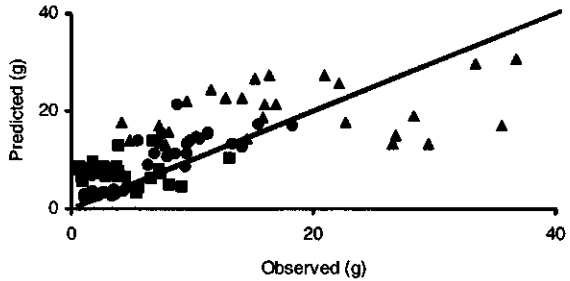
a) Volatile fatty acids (VFA) rumen pool



b) VFA concentration in the rumen liquid



c) Ammonia rumen pool



d) Ammonia concentration in the rumen liquid

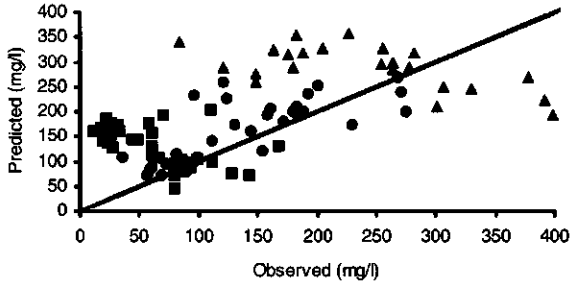


Figure 4. Comparison of observed and predicted values. Observations are: ■, experiment 1; ▲, experiment 2; ●, experiment 3.

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

Ammonia rumen pool exhibited a double peak in the three simulations shown in Figure 5. This pattern was consistent throughout all cows and treatments; the shape of the curve varied with ammonia initial condition, intake rate and composition of the grass ingested. In the model three inputs to the ammonia pool were considered: i) ammonia ingested with the diet, ii) ammonia produced from urea transported across the rumen wall or with the saliva and iii) ammonia produced by fermentation of the soluble protein, in turn arising directly from the feed soluble protein or fermentation of the insoluble, degradable feed protein. Ammonia concentration in the grass was assumed to be 0. An analysis of the inputs during the simulation revealed that the peak of the ammonia rumen pool during grazing was mainly caused by recycling of urea and the peak after grazing by fermentation of soluble protein. The simulated pattern of ammonia rumen pool and ammonia concentration (data not shown) does not agree with our observations (Chilibroste et al., 1999b) nor with previous research under grazing (Rearte and Santini, 1989; Van Vuuren et al., 1986). In the model, the amount of urea transported was related to the concentration of ammonia in the rumen fluid and to the total N intake. There are two main factors that may contribute to incorrect predictions of urea recycling. Firstly, the maximum amount of recycled N per unit of N consumed was set to 0.971 g NH_3-N / g N, representing a potentially high N recycling for the conditions (low N diets fed to Holstein-Zebu cattle) in which the original model was developed; that maximum value may well be too high for temperate grasses containing high levels of N fed to Holstein dairy cattle (Kennedy & Milligan 1980). Secondly, in the original model N recycling is related to N intake in steady state conditions, giving rise to unbiological behaviour in the present discontinuous feeding regimens where the model predicts high urea recycling during eating and no urea recycling during periods when feed intake is zero. Recycling of urea results from urea in saliva and urea transferred by diffusion from the blood to the rumen. Saliva production is stimulated by eating and even more by rumination. Transfer of urea from the blood to the rumen is inhibited by high ruminal ammonia concentrations (Egan et al., 1984). The simulation of the recycling of ammonia is clearly inadequate to represent non-steady state.

In contrast to ammonia, VFA exhibited a smoothed trend which agree with previous research (Chilibroste et al., 1999b): a slow increase of VFA rumen pool at the beginning of the grazing session till the degradation of degradable NDF starts and a maximum rumen pool after the grazing session had finished. However the model assumes that the soluble components of grass ingested are immediately available to the microorganisms to be fermented which maynot be the case (Chilibroste et al., 1998a). Nevertheless the trend followed by VFA rumen pool is well represented and a delay in substrate availability could be easily addressed including a particle dynamics subroutine in the model.

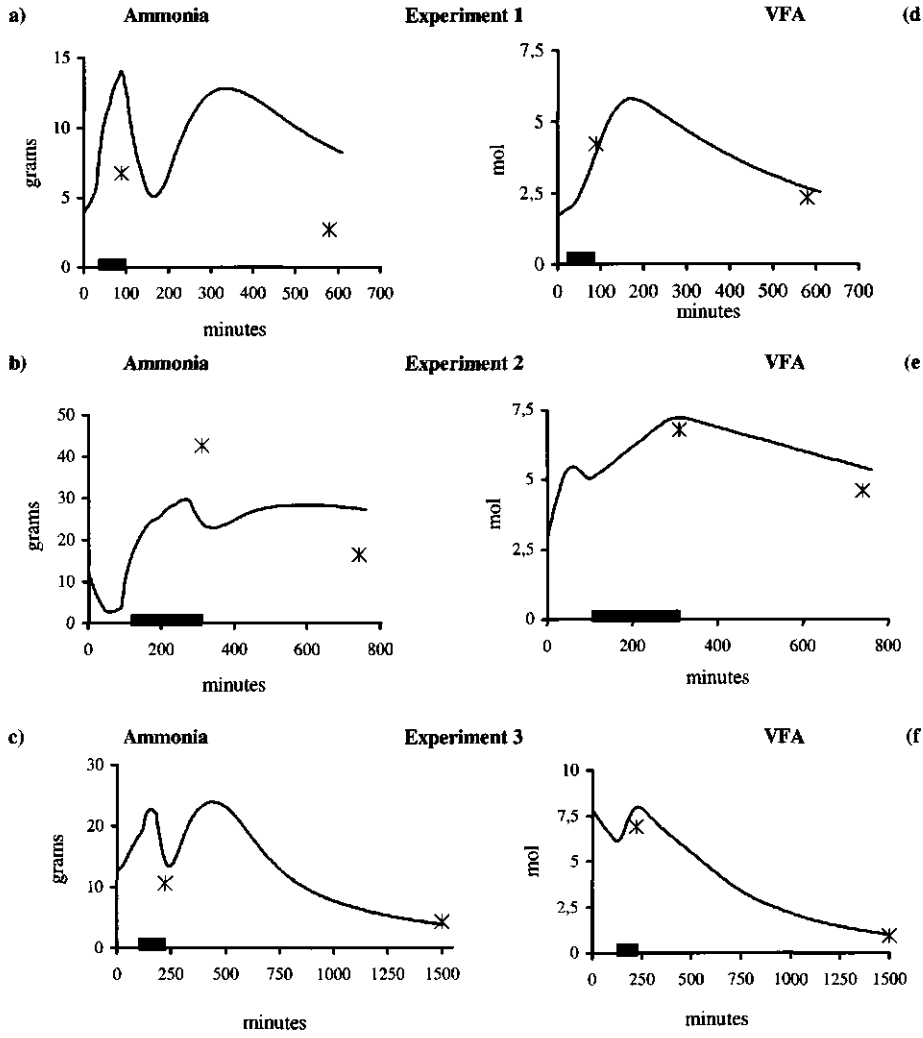


Figure 5. Simulated (solid line) and observed ammonia (plots a, b, c) and volatile fatty acids (plots d, e, f) rumen pools for experiments 1, 2 and 3. Thick solid line represents grazing time (minutes 30 to 90, 100 to 270 and 110 to 180 for experiments 1, 2 and 3 respectively); the observed values after grazing (evacuation 2, Figure 1) and after the starvation period (evacuation 3, Figure 1) are denoted by asterisks.

CONCLUSIONS

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

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GENERAL DISCUSSION

INTRODUCTION

Daily dry matter intake (DMI) may be seen as the summation of individual discrete meals (Forbes, 1995). Under grazing conditions, periods of eating alternate with periods of fasting. For lactating dairy cows two major grazing bouts have been observed: one in the morning and the largest in the afternoon (Rook et al., 1994; Gibb et al., 1997). The research reported in this thesis was focussed in the first grazing bout after a.m. milking and it was based on the large (and somewhat heavy) number of determination planned to be done before and after the grazing session (see experimental protocols Chapters 2 and 5 for details). Sward characteristics, rumen fill and fermentation were considered to be the main candidates to be involved in the control of short term DMI. In order to have individual observations of both grazing behaviour and DMI during the experiments the cows grazed whilst tethered. This method of grazing was extensively used in the past by European dairy farmers (Forbes, 1988), but has been also used in research (Dougherty et al., 1992), and we did not observe any specific problems related to tethering during the experiments. After a short period of training, the cows grazed apparently disinterested of the rope that restrained them and of the surrounding environment.

Under grazing conditions it is generally accepted that DMI is the result of the product of intake rate (**IR**) and grazing time (**GT**) (Laca and Demment, 1996). The discussion of our grazing trials (Chapters 2, 3, 5 and 6) will focus on these two main components of DMI, and the influence of sward and animal characteristics on IR and GT will also be addressed. The gas production technique was used as an *in-vitro* method to characterise the fermentability of rumen contents in animals exposed to different treatments involving periods of starvation, rumen fill and allowed grazing time (Chapter 4). Finally after discussion of experimental results, the perspective of simulation models (Chapters 1 and 7) to contribute to a better understanding and prediction of animal performance under grazing will be considered.

Note about calculations

In this thesis DMI and IR were estimated from changes in DM rumen pools before and after grazing and clearance of DM during the grazing sessions (see diagrams Chapters 2 and 5). Grazing time (GT) was counted as the time elapsed from the moment the cows were placed on the experimental plots until they were removed. To estimate clearance of DM from the rumen two pools were considered: viz. the rumen pool present before grazing and the material being ingested during the grazing session. In all cases, first order kinetics were applied (Robinson et al., 1986). To be able to estimate the losses from the material just ingested, two main assumptions had to be made: first, that the grass was ingested uniformly during the grazing session and second, that the mean residence time of the cleared particles in the rumen was 0.5 GT which could not be necessarily the case (Chapters 2 to 6). Nevertheless, we did not consider that to be of quantitative importance, since clearance of the DM just ingested generally represented a small proportion of the estimated DMI. Even so, to corroborate to which extent a systematic bias may have been introduced by this procedure, a new mathematical approach was used (Dijkstra, personal communication) based on the simple conceptual model shown in Figure 1. According to Figure 1, changes in DM rumen pool size during the grazing session are the result of one input (IR) and one output (clearance of DM out of the rumen) to the pool.

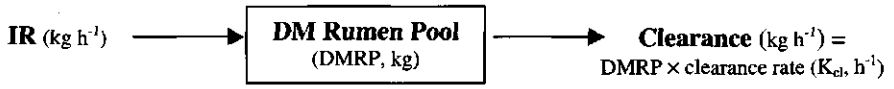


Figure 1. Schematic representation of DM fluxes during a grazing session.

The calculations were performed as follows:

$$\frac{dDMRP}{dt} = IR - (K_{cl} \times DMRP), \quad (1)$$

where:

DMRP = dry matter rumen pool (kg),
IR = intake rate (kg h^{-1})
K_{cl} = clearance rate (h^{-1}), and

Solving differential equation 1 for DMRP yields:

$$DMRP_t = \left[\left(DMRP_0 - \frac{IR}{K_{cl}}\right) \times \exp(-K_{cl} \times GT)\right] + \frac{IR}{K_{cl}}, \quad (2)$$

where

$DMRP_t$ = DM rumen pool after grazing (kg),
$DMRP_0$ = DM rumen pool before grazing (kg), and
GT = grazing time.

Since DMRP before and after grazing, GT and DM clearance rate were either measured or estimated (see Chapters 2 and 5 for detailed methodology), we can solve equation 2 for IR. The new estimation of IR multiplied by GT yields a new estimation of DMI without assumptions about mean residence time of the particles in the rumen. The alternative approaches were compared by regression analysis. The regression of the new calculated values of DMI against the former values of DMI did show slightly higher values for the new derivation ($DMI_{(new)} = 1.06 \pm (0.01) \times DMI$; n=52; RSE = 0.35). Despite the magnitude of the diffferences observed in these experiments, the approach just derived is to be advised, since it avoids unnecessary and probably flawed assumptions. Critical aspects of both approaches remain on the assumption of the material ingested being immediately available for passage

and digestion (Allen, 1996) and the assumption of a common rate of clearance (Okine and Mathison, 1991; Tamminga et al; 1989b).

INTAKE RATE

Substantial progress has been made in the last 10 years in the comprehension of IR and the mechanism underlying the observed responses to changes in pasture characteristics (Laca et al., 1994a;b; Parsons and Chapman, 1998; Ungar, 1996,). The bite has been recognised as the functional link between the animal and the sward (Parsons and Chapman, 1998). The connection between the individual bite and IR can be defined arithmetically: IR over a given time period is the product of the bite mass and mean bite rate over that period (Ungar, 1996).

Bite mass and sward characteristics

The relationship between bite mass and sward characteristics (mainly sward height and density) has been substantially clarified in the last decade at the feeding station level (Laca et al., 1992; 1994a; Ungar, 1996; Newman et al., 1995). Although we did work at a higher level (plot for a complete grazing session), the conceptual framework derived at the lower level was used to analyse the experimental results. In experiment 3, we specifically tested the extent to which sward characteristics per se may impose restrictions on DMI, if mass allowance was not a limiting factor (20.1 ± 4.6 kg DM/cow per grazing session). A significant positive correlation between sward height (SH) and bite mass was calculated ($r = 0.448$, $P<0.05$), tough without a significant linear or quadratic trend (Chapter 5). In this experiment, bite mass was the lowest on measurement day 9 (SH = 6.56 cm) and the largest on day 30 (SH = 27.9 cm). The low SH on measurement day 9 may have prevented larger bite masses (Forbes, 1988; Hodgson, 1985; Laca et al.; 1992; 1994a; Prache et al., 1998) probably due to a low bite depth (Laca et al., 1992) which was not compensated for, bite rate (Laca et al., 1994a), nor by density of the grazed horizon (Wade, 1991). However, bite mass tended to be larger on measurement day 6 (SH = 6.18 cm) than on day 9 even though SH and sward density were lower on day 6. A relatively large bite mass has been observed in very short swards (Rook et al., 1994) associated with a shorter grazing session. Since in these experiments pasture allowance was not limiting DMI, on measurement day 6 the cows had a large area to explore (299 m^2) and from which to select bites. If the animals could select the larger potential bite first and decided to stop the grazing session before this grazing horizon had been completely depleted (Laca et al., 1994b), they will realize a good bite mass and IR, even though it is only sustainable for a short period of time. An extension of the grazing time under similar SH as did occur on day 9, will necessarily be at the expense of a lower bite mass and IR. To which extent the large GT observed on measurement day 9 was a consequence of the small bite mass realized on that day, or the small bite mass observed was the product of a long grazing session, is not clear from our results. The rain conditions on day 9 may have contributed to the small bite mass. It has been suggested that surface moisture and lubricity of the laminae might increase slippage between the incisor and dental pad resulting in a smaller bite mass (Gibb et al., 1998). Low DM content of feed has also been suggested as a potential constraint to DMI in cattle (Tamminga and Van Vuuren, 1996) and sheep (John and Ulyatt, 1987), but this phenomenon has not been investigated extensively (Ungar, 1996).

Bite mass and animal characteristics

To minimise possible constraints to DMI from the sward characteristics, an ample allowance of pasture was offered to the cows in experiments 1 (30.2 ± 3.7 kg DM) and 2 (23.7 ± 2.5 kg DM) per cow per grazing session. Therefore, in these experiments we did expect to explain variation in bite mass by the treatments imposed to the animals and not by sward characteristics. In experiment 2 the factors length of starvation and rumen fill before grazing were investigated. The length of starvation before grazing (2.5 and 16.5 h) did not significantly change bite mass, which is in agreement with previous research (Greenwood and Demment, 1988). It seems that starvation before grazing may induce a larger bite mass at the beginning of the grazing session (experiment 1), but it was a transient effect since bite mass declined as the grazing session progressed. A reduction of bite mass with grazing time has been explained by the depletion of the grazing horizon and by the overlapping in subsequent bites of ungrazed and already grazed vegetation (Distel et al., 1995; Laca et al., 1994a). The inclusion of inert rumen material within the rumen did reduce bite mass significantly mainly in the short time-period (2.5 h) starved animals. The explanation for this observation was not immediately obvious. It must be born in mind that we used polystyrene material as the inert rumen bulk that, in addition to its fill effect, may have stimulated the tactile receptor of the rumen wall and thus initiated pseudo-rumination behaviour (Baumont et al., 1990).

A general correlation and regression analysis performed throughout the three experiments (n=52) did not show any significant correlation between bite mass and rumen pool sizes before grazing (total, DM, NDF, ADL, NH3 and VFA). The lack of a significant relationship between starvation or rumen fill, and bite mass supports the hypothesis that for a given animal, bite mass is more linked to sward than to animal characteristics. Bite mass is be a function of the initial sward characteristics and whether it can be maintained with time will depend on the pasture depletion process.

Bite mass and bite rate

Average bite rate did not change significantly in our experiments (Chapters 2 and 5). Cows started the grazing bouts at a high bite rate rather independent of the initial sward (experiment 3) or animal (experiment 2) conditions. These initial, high bite rates were probably the result of the period of starvation (Greenwood and Demment, 1988), and were most likely induced by a feeling of hunger exacerbated by the rumen emptying procedure prior to grazing. Although field studies have generally found an increase in bite rate as bite mass declined (Hodgson, 1985; Forbes, 1988; Pratche 1998), data from our experiments did not indicate a significant relationship between these two variables. Bite mass and bite rate are not independent, the most obvious functional link being the fact that larger bites would require more ingestive mastication. However Laca et al. (1994a), have shown that bite rate did not necessarily increase with bite mass since the cows are able to overlap biting and chewing jaw movements. This indicates the existence of a critical bite mass below which, time per bite and total number of chewing movements does not change. Although not directly comparable with the experiment of Laca et al. (1994a), bite mass in this study (0.4 to 0.97 g bite^{-1}) was within the range in which the overlapping mechanism was effective (0.5 to 1.5 g bite^{-1}, Laca et al.,

1994a). Nevertheless bite rate was reduced during the grazing sessions as shown in Chapter 5. During the first hour of grazing, the changes in BR were not significant, but declined significantly ($P<0.01$) thereafter. Considering the three experiments together, a significant negative correlation ($r = -0.41$; $P < 0.01$; $n=52$) was found between average bite rate and grazing time. This would suggest that the cows spent more time per bite as the grazing session progressed. However, the correlation between bite rate and eating time (the time during which the cows were effectively biting) was not significant, suggesting an increase in the intra-meal intervals (Gibb, 1998) or searching time, but not in time per bite.

Defoliation process

Bite mass is the result of bite depth, bite area and sward density of the grazed horizon (see Figure 1 of General Introduction). Bite depth has a major implication for the paddock utilisation as a whole since it will determine the stubble sward height that is a key factor either for regrowth or for further grazing. Although the influence of sward characteristics for bite depth has been recognised and quantified (e.g. Laca et al., 1992; Ungar et al., 1991), the optimum bite depth given certain sward conditions is still a matter of debate. The hypothesis that the animal removes a relatively fixed proportion of the total height of the vegetation is gaining acceptance (Demment et al., 1995; Parsons and Chapman, 1998). An "horizon" grazing style has been proposed for steers (Laca et al., 1994b) and dairy cows (Wade, 1991) grazing ryegrass. Parsons and Chapman (1998) suggested this grazing strategy as the most cost-effective, where the animal takes as large bite as possible, provided that this does not compromise the capacity to masticate or ruminate it. In this sense, the pseudostems height may be have an important role in determining bite depth (Arias et al., 1990; Flore et al., 1993; Illius et al., 1995). Figure 2 shows, sward height removed expressed as a percentage of sward height present before grazing. A simple exponential model (diminishing return type) with a threshold minimum sward height was fitted to the observed data (Figure 2).

It is noteworthy that the asymptote estimated by the model (28.6 %) was close to the value observed by Wade, 1991 (32 %), who also worked with lactating dairy cows grazing ryegrass. The asymptote predicted by the model in Figure 2 should underestimate bite depth, since we worked with an ample mass allowance, and sward height after grazing was an average of grazed and un-grazed areas. The model of Figure 2 also estimates a minimum threshold level (2.9-cm) below which, either the animals are not able (physical barrier) or refuse (behavioural constraint) to graze. If this grazing pattern by horizons could be confirmed as systematic, the reduction in IR during the grazing session could be predicted simply from the reduction in bite mass as the initial grazing horizon is depleted and a second "residual" horizon becomes available.

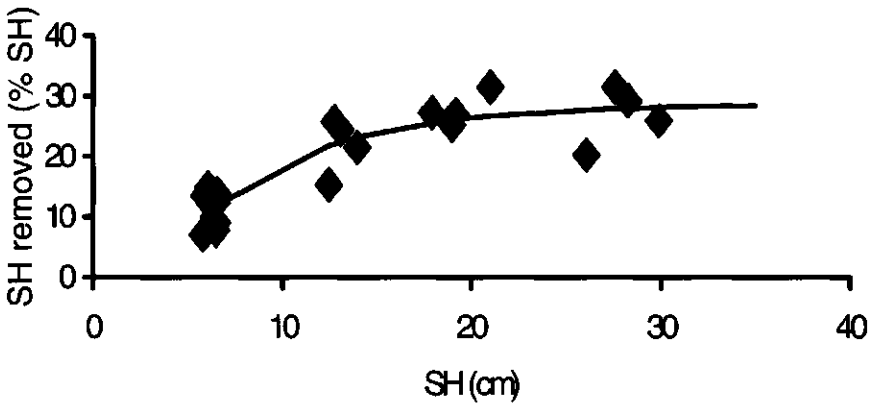


Figure 2. Sward height removed (SHR, %) as a percentage of sward height determined before grazing (SH, cm). Model: SHR = 28.6 ±(2.6) × exp (-0.15 ±(0.06) × (SH-2.96 ±(1.4))), RSE= 3.79.

GRAZING TIME

In contrast to the significant progress that has been made on IR, control of GT still remains poorly understood. The following statements from authorities in the grazing field clearly express the lack of information concerning the ultimate control of grazing time: "...there has been little progress in providing a mechanistic account of the factors controlling grazing time, even though this is essential to progress from an understanding of instantaneous intake rate to understanding daily intake." (Parsons et al., 1994), "...there is currently no known physiological mechanism that adequately explains grazing time." (Newman et al., 1995), "...prediction of grazing time has been a fundamental but elusive goal of intake models." (Laca and Demment, 1996). This gap of knowledge has also hampered the development of mechanistic models able to predict DMI or even to translate to daily DMI, the fundamental relationships established for IR control mechanisms (e.g. Ungar, 1996).

In this thesis the main aspects investigated as influencing GT were: i) the effect of rumen fill and length of starvation before grazing (Chapters 2 and 3) and ii) sward characteristics when allowance was set as not to limit DMI (Chapter 5and 6). Moreover, the detailed information reported in Chapters 2 to 6 offer a reasonable large data set to explore possible constraints to GT at ruminal level, including rumen fill and kinetics, rumen fermentation end-products, pH, and osmotic pressure.

Grazing time and animal status

Effects of fasting on IR and DMI has been studied previously in sheep (Jung and Koong, 1985; Edwards et al., 1994; Newman et al., 1995) and cattle (Greenwood and Demment, 1988). During fasting, the metabolism of the animal changes since nutrient input is absent, and catabolic processes must start to cover basic maintenance requirements. In the rumen itself, a number of important changes take place. Fermentation and passage of particles are both continuous processes. Therefore, the fasted animal faces a progressively emptier and somewhat depleted rumen compared with the non-fasted animal (Chapters 2 and 3). Experiment 2 was designed to separate the effect of fasting from the effects on rumen fill on the internal status of the animal. In a 4x4 Latin Square design a 2x2 factorial arrangement of treatments was randomised, comprising two lengths of starvation period and the presence or absence of synthetic indigestible material in the rumen before grazing. Starvation as such had a significant effect on grazing time, but the magnitude of the effect tended to vary depending on whether or not bags were present in the rumen (Table 3, Chapter 2). The inclusion of indigestible material in the long-starved cows led to a reduction in grazing time of 44 minutes, while in short-starved cows this reduction was only 13 minutes. The tendency towards an interaction between the duration of starvation and the amount the indigestible material in the rumen, support the hypothesis of additive effects of treatments on grazing time control (Forbes, 1995; Gill and Romney, 1994; Mbanya *et al.*, 1993). Nevertheless, it must be born in mind that the polystyrene material used to simulate rumen fill, in addition to their fill effect, may have stimulated the tactile receptors of the rumen wall and thus initiated rumination behaviour (Baumont *et al.*, 1990).

Grazing time and rumen fill

From the early sixties, the capacity of the digestive tract and especially that of the rumen, has been addressed as an important constraint to DMI in forage-fed ruminants (Campling and Balch, 1961; Conrad, 1966; Freer and Campling, 1963). Although the hypothesis has received substantial criticism (e.g. Grovum, 1987; Ketelaars and Tolkamp, 1991) the role of rumen fill as a DMI constraint is still under investigation (e.g. Bosch, 1991; Dado and Allen.; 1995; Gassa et al., 1991; Johnson and Combs, 1992). Moreover, rumen fill has been incorporated into feeding evaluation systems (e.g. Mertens, 1994; NRC, 1988) to constrain the predicted maximum DMI. The rumen fill theory has been supported by three types of experimental evidence (Faverdin et al., 1995): i) the presence of stretch and mechano-receptors in the rumen wall; ii) the effect on DMI of addition of material to the rumen; iii) the relationship between certain feed attributes (typically cell wall content and DM digestibility) and DMI.

The first problem to be addressed in assessing the importance of rumen fill, is to specify which, if any, fraction might properly represent rumen fill. For daily DMI regulation, NDF in the feed has been suggested as the best predictor of rumen fill (Mertens, 1987; 1994). Van Soest et. al. (1991) established that NDF is more closely related to the daily ruminating time, rumen fill and DMI, than other chemical fractions like crude fibre and acid detergent lignin. The integration of fibre dynamics in a simulation model aimed to predict daily DMI (Chapter 1) showed good correlation between predicted and observed DMI. Nevertheless when balloons were introduced in the rumen, DM rumen pool has been normally selected as an indicator of rumen fill (Faverdin et al., 1995). In detailed studies of digestion kinetics (e.g.

Bosch, 1991; de Visser, 1993; Robinson et al., 1987; Van Vuuren, 1993) and particle breakdown kinetics (Waghorn, 1986; Waghorn et al., 1989) the total rumen content as well as the chemical components have also been considered. Table 1 shows the correlation between total, DM, N, NDF and ADL rumen pool sizes observed in our experiments after grazing. All pool sizes were positively, and significantly correlated to each other.

Table 1. Correlation between rumen pool sizes after grazing for the three experiments (n= 52)

	Total (kg)	DM (kg)	NDF (kg)	ADL (kg)	N (kg)
Total (kg)	1.00	0.92***	0.91***	0.81***	0.77***
DM (kg)		1.00	0.95***	0.90***	0.88***
NDF (kg)			1.00	0.83***	0.71***
ADL (kg)				1.00	0.87***
N (kg)					1.00

***, $P<0.01$

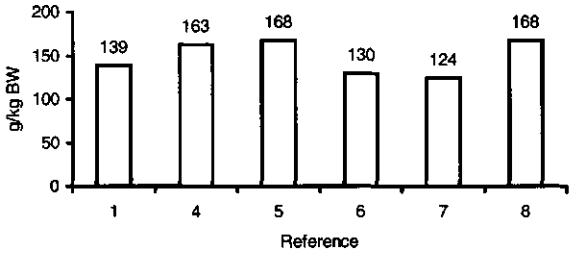
To determine whether or not rumen fill (total, DM or NDF) might have played a role in GT control, the observed values after grazing are presented in Figure 3. Treatments were removed from the calculations where GT was intentionally interrupted (treatments 1 and 2 of experiment 1), where inert material was introduced in to the rumen (treatments 1 and 3 of experiment 2), or where there was low sward height (< 10 cm) before grazing (measurement days 6 and 9 of experiment 3). Therefore, only those observations where the rumen was likely to be directly involved in GT control remained in the analysis. To put our observations into perspective, they were compared with values reported for dairy cattle fed fresh ryegrass indoor (Van Vuuren et al., 1992), grass silage differing in maturity (Bosch et al., 1992), a mixture (50:50) of grass and corn silage plus concentrate (de Visser et al., 1992) and lucerne hay (Hartnell and Satter, 1979). Additionally, the experimental results were compared with other bibliographical sources.

In our experiments the DM rumen pool size after grazing was higher than the rumen pool observed by Van Vuuren et al. (1992), but close to the figures reported by Waghorn (1986) and Waghorn et al. (1989), working with fresh lucerne and ryegrass. It must be noted that Van Vuuren et al. (1992) worked with a younger ryegrass than here, as indicated by the higher N and lower NDF and DM content compared with our figures. However the observed DM rumen pools sizes were smaller than values reported for diets including high proportion of concentrates (> 40%) eg. Bosch et al. (1992); Dado and Allen. (1995), De Visser et al. (1992) and Shaver et al. (1986; 1988). The differences seem to be larger when the NDF rumen pool sizes are compared (see plot c, Figure 3). Nevertheless, regarding the total rumen content pool sizes (plot a, Figure 3) the relative differences were smaller (reference 1 vs. 5 to 8, Figure 3) than the differences in DM or NDF pool size. Especially close, is the comparison with the total rumen pool sizes reported by de Visser et al. (1992). Unfortunately, Van Vuuren et al. (1992) did not report the total, or the DM percentage of the rumen contents.

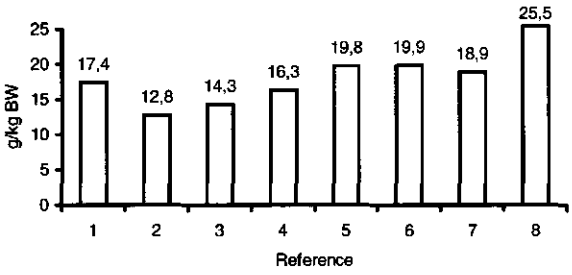
Nevertheless, it seems that cows fed fresh grass had problems not "to accommodate a large volume" of material in the rumen but to "pack it" properly. Since the difference between plots (a) and (b) of Figure 3 are mediated by the DM percentage of the rumen contents, the relationship between DM percentage, DM rumen pool and total rumen pool in experiments 1, 2 and 3 was investigated and the results are shown in Figure 4 and 5.

From Figure 4 it seems that the cows can not increase rumen content DM percentage over 12.5 %. A practical consequence of this observation is that when a certain DMC threshold level is approached the only possible alternative for the cows to increase rumen content is by increasing rumen volume. Besides, the logistic model fitted in Figure 5 suggests that changes in rumen volume at a low rumen content are mainly explained by changes in the liquid fraction, with an asymptotic DM rumen pool of 0.76 kg as total rumen pool approaches 0. Dardillat and Baumont (1992), reported DM percentage of the reticulum content as 5% for cows fed fresh grass. Chilibroste et al. (unpublished) determined an average value of 3.4 % for the liquid fraction of cows fed fresh grass. The logistic model suggests an exponential increase up to a certain level (approximately 75 kg in this research), thereafter increasing at a diminishing rate to reach an asymptote of 15.4 kg DM in the rumen. The significant positive correlation ($r = 0.92$; $P<0.01$; $n=52$) between total and DM rumen pool size for the whole data set (Table 1) was consistent with this observation. The low DM content of the forage fed to the cows (Van Vuuren et al., 1992; Waghorn, 1986; Waghorn et al., 1989;) may play a role in the low DM percentage observed. De Visser et al. (1993), working with a mixture of wet material (33-39 % DM) also reported rumen DM percentages of 12 to 13 %. However, the silage fed by Bosch et al. (1992) had a DM content over 45 % and the DM percentage of the rumen content was still 10 to 11 %. A close relationship between water intake with the feed (29.1 ± 10.9 L) and the changes in non-DM rumen pools sizes (26.2 ± 12.6 L) was observed in experiments 1 and 2, in agreement with results of Waghorn (1986). Nevertheless the question: why the cows cannot quickly get rid of this water, still remains. One possible explanation may be a low chewing efficiency (particle size reduction) during eating exhibited by the fasted grazing cows. For fresh forages, chewing efficiency during eating may be more influenced by the rate of eating, than by the type of feed (Waghorn, 1986). Under grazing conditions, it has been shown that the cows are able to reduce chewing during eating to increase IR (Parsons et al., 1994; Laca et al., 1994a), especially after a period of fasting (Greenwood and Demment, 1988).

a)



b)



c)

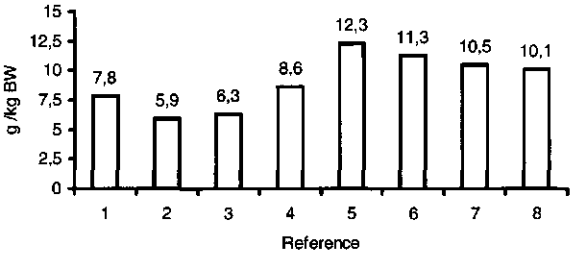


Figure 3. Total (a), DM (b) and NDF (c) rumen pool sizes in grams per kg of body weight (g / kg BW).
Reference:
1, Chilibroste et al, this Thesis (n=28),
2 and 3, estimated from Van Vuuren et al. (1991),
4 and 5, adapted from Bosch et al. (1992),
6 and 7, adpated from de Visser et al. (1992), and
8, adapted from Hartnell and Satter (1979).

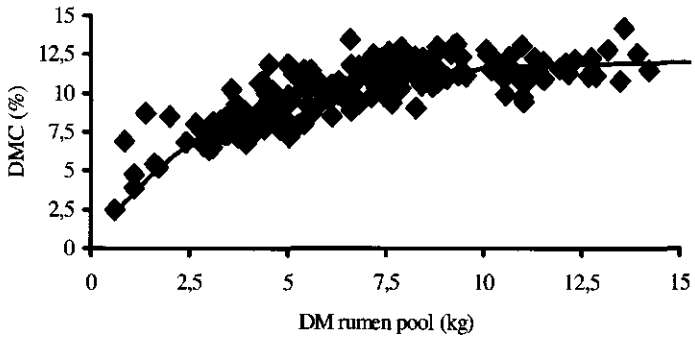


Figure 4. Observed and predicted DM content (DMC, %) in the DM rumen pool (DMRP, kg) for experiments 1, 2 and 3 this Thesis. Symbols denote observed values; the solid line represent the predicted values.
Model: DMC = 12.05 ± (0.189) × exp (-0.32 ± (0.17) × DMRP); RSE = 1.24.

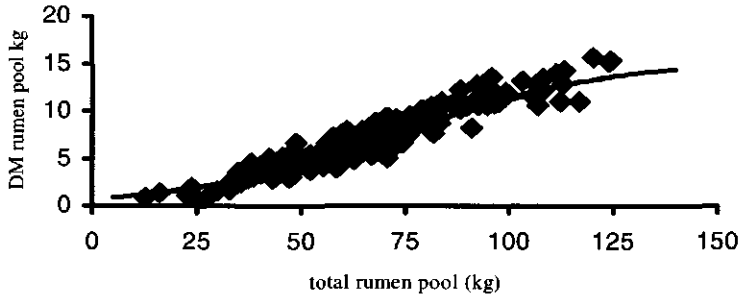


Figure 5. Relation between DM rumen pool (DMRP, kg) and total rumen pool (TOTRP, kg) for the experiments 1, 2 and 3 this Thesis. Symbols denote observed values; the solid line represent the predicted values.
Model = DMRP = 15.4 ± (0.68) / (1+19.0 ±(2.1) × exp (-0.04 × TOTRP)), RSE=0.71.

The particle size distribution of rumen contents after grazing (reported in Chapter 3) supports this hypothesis: after 1 hour of grazing, approximately 75% of the DM ingested was over 1.25 mm.This figure is larger than that reported for animals fed indoors (Ulyatt et al., 1986; Waghorn, 1986). This aspect of ingestion under grazing requires further research and clarification because of the practical implications it may have for feeding strategies in intensively managed grazing dairy systems. It must be remember that in grazing dairy systems, fasting does occur normally (Gibb et al, 1997; Rook et al., 1994). Moreover, in grazing systems where the cows may have to walk one or more km to reach the paddock (e.g. dairy systems of Uruguay and Argentina), periods of fasting of 3 to 5 hours are not

uncommon. Otherwise in grazing systems where daily strip grazing is the dominant grazing system, a fast depletion of the available pasture occurs. Under these conditions, the dairy cows are "pushed" to behave as though "fasted" as an effective way of competing for a scarce resource and to realise the highest possible IR (Chilibroste et al., unpublished).

It is generally accepted that the inclusion of concentrates in the diet is the more effective way to increase DM content and therefore DM rumen pool (Bosch, 1991, Gassa et al., 1991). Nevertheless, alternative approaches under grazing conditions aimed to improve efficiency of ingestion and digestion, have not been explored yet and merit further research.

Grazing time and fermentation end-products

When cattle were fed diets consisting of fresh ryegrass and white clover, a large proportion (0.85 to 0.95) of the OM ingested was fermented in the rumen (Cammell et al., 1983). Since volatile fatty acids (VFA) and ammonia (NH_3) are the main fermentation products, they have received special attention as candidates to be involved in DMI control in ruminants (Forbes, 1995; Gill et al., 1988; Grovum, 1987; 1995; Leuvenink, 1998; Van Os, 1997). Van Vuuren (1993) also suggested that in dairy cows fed fresh grass, VFA and NH_3 concentrations might have played a role in DMI regulation. In Table 2, some fermentation parameters (and mutual correlation's), observed at the end of the grazing sessions in experiments 1, 2 and 3 are shown. As in the previous section, those treatments were removed where GT might have been stopped by the inert rumen bulk introduced in the rumen (experiment 2) or by a short sward height (experiment 3). Treatments 1 and 2 of experiment 1 were also removed, since the grazing session was intentionally interrupted before a voluntary grazing end was reached (28 of the 52 observations remained for calculations).

Table 2. Volatile fatty acids (VFA) concentration (mmol L^{-1}) and pool (VFAP, mol), ammonia (NH_3) concentration (mg L^{-1}) and pool (NH_3P, g), acetic to propionic ratio (C_2/C_3), glucogenic to non-glucogenic ratio (NGR) and nitrogen to organic matter ratio (N/OM, g/kg) in the rumen after the grazing session.

	VFA	NH_3	VFAP	NH_3P	PH	C_2/C_3	NGR	N/OM
Mean±SE	88±20	206±135	6.1±1.7	16±14.2	6.47±0.3	3.2±0.7	4.4±0.8	41±5.9
VFA	1.00	-0.32*	0.63***	-0.27	-0.55***	-0.54***	-0.50***	-0.32*
NH_3		1.00	0.30	0.96	0.45**	0.82***	0.78***	0.03
VFAP			1.00	0.46**	-0.31	0.03	0.07	-0.27
NH_3P				1.00	0.36*	0.79***	0.76***	-0.00
PH					1.00	0.27	0.27	0.34*
C_2/C_3						1.00	0.97***	-0.17
NGR							1.00	-0.12
N/OM								1.00

SE, standard error; NGR, (acetic + 2 butyric)/propionic (mol/mol).
*, $P<0.1$; **, $P<0.05$; ***, $P<0.01$

In general, at the time the cows stopped grazing, rumen concentrations and pool sizes were within physiological ranges (Bergman, 1990). Higher values of VFA and ammonia concentration have been observed in grazing lactating dairy cows (Mattiauda et al., 1997; Rearte and Santini, 1989; Van Vuuren et al., 1986). It does not seem evident from the mean

figures of Table 2 that the concentration of any of these metabolites individually may be triggering the end of the grazing session in our experiments. Nevertheless there are some points that deserve further attention:

1. In experiment 1, the similarity between VFA rumen pool before and after 1 hour of grazing (Chapter 2) suggests a delay in the fermentation process and support the suggestion that during eating, the chewing efficiency of fasted animals grazing ryegrass may be low.

2. Although for these three experiments the mean ratio between acetic and propionic acid rumen pools (C_2/C_3, Table 2) may be considered within a normal range, in experiment 2 (Chapter 3) the grazing session finished with a C_2/C_3 ratio of 4.5 and 3.6 for long and short starved cows respectively. Mbanya et al. (1993) found a depression in DM intake after infusion of either acetic (C_2/C_3 = 5.0) or propionic (C_2/C_3 =1.65) acids, but not when both were infused (C_2/C_3 = 2.86), which indicates that the C_2/C_3 relationship may be more relevant as a signal of satiety than the absolute values of each one individually. In experiment 2, the long starved cows tended to stop grazing before full ruminal capacity was reached and the larger imbalance of nutrients on these animals may have been involved in this behaviour. The importance of imbalance of nutrients on DMI has been recently addressed (Illius and Jessop, 1996; Poppi et al., 1994) and might be especially relevant for animals fed "imbalanced diets" as high quality fresh grasses and legumes are (Beever and Siddons, 1986; Van Vuuren, 1993).

3. The VFA and NH_3 concentration patterns reported in experiment 3 (Chapter 6) showed that: firstly, the maximum concentration of all the metabolites was observed after the grazing session was finished (20 to 100 min), and secondly that the NH_3 peak ocuured before that of VFA. By definition, if an animal will stop the grazing session triggered by a threshold level of any metabolite, this level must occur before the end of the meal.

Grovum (1987) suggested that VFA's could contribute to satiety through changes in rumen fluid tonicity. Grovum (1995) also stated that, if any, effects of VFA in the rumen on satiety must be mediated by changes in hypertonicity. In experiment 3, we did measure osmotic pressure sequentially in the rumen fluid, but the observed values at the end of the grazing session (Chapter 5) were lower than the values that depressed DMI in sheep (400-500 mosmol L^{-1}) (Grovum, 1995).

However it must be borne in mind that for either VFA (Faverdin et al., 1995; Forbes, 1995) or osmotic pressure (Grovum, 1995) a dose-response relationship has been observed between level infused and DMI depression which suggest that small increments from the baseline level should induce a reduction in DMI.

Clearance rate

Under steady state conditions feed intake equals clearance from the rumen, which is the sum of microbial degradation and passage (Tamminga et al., 1989a). Therefore energy supplied by the feed is related to rumen fill on the one hand, and to rumen clearance on the other (Tamminga and Van Vuuren, 1996). Rumen outflow is considered to follow first order kinetics (Robinson et al., 1986), which means that per unit of time a rather constant fraction of what is present is cleared from the rumen by passage to the lower gut.

In experiments 1, 2 and 3 we did estimate clearance rate (k_{cl}) for DM, OM, NDF and ADL from changes in rumen pool sizes during the periods of starvation. In experiment 1 and 2 (long-starved cows), k_{cl} was estimated before and after the grazing session (see diagrams of Chapters 2 and 3) while in experiment 3, k_{cl} was estimated only after grazing. The pooled results are presented in Table 3.

Table 3. Estimated clearance rate (mean ± SE; % h^{-1}) for dry matter (DM), neutral detergent fibre (NDF) and acid detergent lignin (ADL).

	Before grazing (n=24)	After grazing (n=52)	Difference (n=24)
OM	6.07±0.73	7.23±1.77	1.58±1.55, $P<0.01$
NDF	5.40±0.79	6.43±1.75	1.58±1.82, $P<0.01$
ADL	3.70±0.85	3.91±1.71	0.23±1.82, NS

The estimated clearance rate (k_{cl}) values of OM and NDF were within the range estimated by Bosch (1991) for grass silage differing in maturity, but lower than the values reported by Van Vuuren et al. (1993) for fresh ryegrass differing in growing season and fertilisation at the time of harvesting. We use clearance of ADL as an indicator of passage rate although accuracy might be low (Tamminga et al., 1989a). The observed k_{cl} values of ADL were within the ample range of passage rates estimated by Owens and Goetsch (1986), Poncet et al. (1995) and Sauvant et al. (1995) for forages. Van Vuuren et al., (1992; 1993) working with fresh ryegrass reported lower values of passage rate when it was estimated using lignin as a internal marker (2.4 – 3.5 % h^{-1}). Mambrini and Peyraud (1994) also reported lower values of passage for fresh ryegrass labelled with rare earth metals. In general, we did not detect significant differences between treatments. A large residual variation within experiments was always present probably due to the utilisation of ADL as an internal marker (Tamminga et al., 1989a). If we do assume that approximately 70 % of the OM and NDF rumen pools were insoluble but potentially degradable (Chapter 4), the estimated fractional degradation rates for OM and NDF were 3.4 and 2.4 % h^{-1} before grazing and 4.7 and 3.6 % h^{-1} after grazing. The estimated fractional degradation rates after grazing are lower than the figures estimated by Van Vuuren et al. (1992; 1993) for fresh ryegrass. The degradation rates estimated before grazing (overnight starvation) seems to be too low for vegetative fresh ryegrass, but to a certain extent may be the result of an overestimation of passage rate. Tamminga and Van Vuuren (1996) pointed out a number of aspects that characterise passage and microbial degradation rates, each of which could be a rate-limiting factor.

A general correlation analysis throughout experiments showed that k_{cl} of OM, NDF or ADL either before or after grazing was not significantly correlated with any indicator of rumen fill (total, DM, NDF, ADL rumen pool size) or rumen chemical composition. Bosch (1991) also observed a lack of correlation between k_{cl} and rumen fill. Interestingly the only significant correlation ($r = 0.43$; n=26; $P<0.05$) was between k_{cl} of ADL and DM % of the rumen content after grazing. Tamminga et al., (1989b) and Allen (1996) suggested a relationship between degradation and passage rate, since the potentially fermentable particles would require a certain level of degradation before they are able to increase the functional specific density and reach the reticular omasal orifice. Again it is possible that particle kinetics i.e., particle size,

position and ratio between potentially degradable and undegradable fractions, may have played a role in the observed differences. The requirement for a better understanding and more accurate quantification of passage of particles out of the rumen has been pointed out by Tamminga et al. (1989b) and Dijkstra and France (1996).

The differences between OM and NDF clearance rate before and after grazing are noteworthy. A number of factors may have been involved in the observed differences: i) existence of different fractional rates during the day at eating, ruminating or resting (Okine and Mathison, 1991), ii) higher passage of small particles with a lower degree of fermentation due to the more intense rumen wall contractions at the onset of eating (Dardillat and Baumont, 1992), iii) the large amount of fluid influx with the feed (Owens and Goetsch, 1986), iv) the different length of time involved in both estimation or a combination of them. This fact deserves further attention in research as well as in mechanistic rumen model development (Dijkstra and France, 1996).

THE USE OF CUMULATIVE GAS PRODUCTION TECHNIQUE TO CHARACTERISE RUMEN FERMENTABILITY

The measurement of cumulative gas production (GP) has been proposed as a simple and accurate *in vitro* method to study the fermentability of feeds (Beuvink, 1993; Pell and Schofield 1993; Theodorou *et al.* 1994). Good correlation between GP parameters and *in vivo* measurements such as DMI (Blümmel and Ørskov, 1993; Blümmel and Becker, 1997) or *in vivo* digestibility (Blümmel and Ørskov, 1993; Khazaal *et al.*, 1993) have been found. New automated equipment has been developed to measure gas production (Cone *et al.*, 1994; Davies *et al.*, 1995) so that more accurate gas production profiles are being generated, in which different underlying processes can be identified (Groot *et al.*, 1998) and the fermentative behaviour of soluble fractions isolated and described (Cone *et al.*, 1997; Schofield and Pell, 1995; Stefanon *et al.*, 1996). These recent developments make GP a promising technique to improve understanding of rumen fermentation kinetics. In Chapter 4 we used the cumulative GP technique to characterise changes in fermentation of rumen contents that result from the treatments applied in experiments 1 and 2.

Firstly we checked whether or not the apparently lower chewing efficiency exhibited by the cows after a period of starvation at the beginning of the grazing session was reflected in fermentability parameters. The rumen samples after 1 hour of grazing had significantly higher values of cumulative gas production and lower half-times than the cows that grazed longer (Table 3, Chapter 4), despite the lower DMI and DM rumen pools of the cows that grazed for 1 h.. To test the hypothesis that the observed differences in gas production may have been due to a larger proportion of intracellular soluble content, we fitted a multiphasic logistic model. We expected different fermentation patterns that would represent different substrate fractions (Groot et al., 1998; Schofield and Pell, 1994), but this was not the case. The high dilution of the inoculum on the GP systems used at WAU and the use of a manual reading that only allowed few points in the first hours of incubations may have hampered a better fitting of the multi-phasic model.

Secondly we correlated the GP parameters with rumen contents and chemical composition. Cumulative gas production was significantly correlated with the ADL content of the rumen

samples, but not with the NDF content. However, NDF was positively correlated with the time required to produce half of the total gas, giving an indication of the sharpness of the gas production profile. The amount of gas produced per g OM fermented was negatively correlated with the N content of the rumen samples as was observed by Chilibroste *et al.* (1998) for grass samples. Menke and Steingass (1988) have suggested that CO_2 can be captured by NH_4 and from NH_4HCO_3, leading to an underestimation of the amount of gas produced. Also, Blümmel and Becker (1997) found that more gas was produced from 200 mg NDF than from 200 mg whole roughage and suggested the efficiency with which the fermentable material is incorporated into microbial cells as an explanation for the higher gas volume from NDF.

It can be concluded that measurement of fermentation kinetics by cumulative gas production was suitable to detect changes in rumen content fermentation patterns due to the clearance of rumen content (effect of starvation) or DMI during the grazing sessions. The GP parameters were more related to the rumen content chemical composition than to the rumen pool size or kinetics. The use of the GP technique to characterise nutrient availability to ruminal micro-organism needs further research.

Perspectives in the use of Modelling to Predict DMI and Availability of Nutrients to Dairy Cows.

As discussed in the previous sections the "pasture–animal" interface involves interactions between a large number of variables. The interaction of animal and sward variables create a complex system and from this perspective an interest in modelling is not only justified, but it may represent the only way to accommodate the complexity of the system. A proper balance between modelling and analytical research has been proved as an effective strategy to improve both understanding and quantification of complex systems (Demment et al., 1995; Dijkstra and France, 1995). Significant progress has been made in the last decades in modelling foraging strategies (e.g. Dove, 1996; Laca and Demment, 1996), forage quality (e.g. Illius and Allen, 1994) and whole rumen function (Bannink et al., 1997; Dijkstra and France, 1994), but, unfortunately almost without making the crucial connection between the different fields. For the foraging models the digestion and absorption of nutrients has frequently been ignored (Laca and Demment, 1996) and in the whole function rumen models DMI is a defined and often a continuos input (Dijkstra and France, 1996).

Although many integrated models have been developed, only a few were developed to predict voluntary DMI. No doubt the limitations of the extant models either representing or predicting key processes, like diet selection under grazing, particle kinetics in the gastrointestinal tract, microbial population dynamics and production and absorption of fermentation end-products (see reference previous paragraph), have hampered further development. Additionally it is not known yet how the different stimuli received by the animal are integrated to modulate eating behaviour (Forbes, 1995). Nevertheless, recent attempts to integrate physical and metabolic signals controlling DMI (Fisher, 1996, Poppi et al., 1994, Sauvant et al., 1996) and the ingestion and digestion under grazing as a unique process (Laca and Demment, 1996; Newman et al., 1995), are noteworthy and challenge further developments.

Regarding the problem outlined in the General Introduction of this thesis, a major limitation of the extant models to evaluate and improve feeding strategies, is their assumed steady state conditions. Additionally, these models evaluate one feed at a time (either an individual feed or a complete mixture of different feeds) and little attention has been given to the construction of models with a combination of two or more feeds fed separately. The need for models which represent discontinuous feeding regimes, and predict rumen pools varying in size with time, has been highlighted (Dijkstra and France, 1996) and seems to be essential to represent the unique process of ingestion and digestion under grazing. In this Thesis two different approaches were followed. Firstly, a mechanistic simulation model with a flexible construction was developed to be able to operate with different feeds fed under discontinuous feeding regimes (Chapter 1). Secondly, a dynamic simulation model of digestion and absorption of nutrients (Dijkstra et al., 1996) was modified and evaluated under non-steady state conditions (Chapter 7).

The model described and evaluated in Chapter 1 was developed in the earliest stages of this research program to predict DMI. The model has a number of remarkable characteristics: i) it allows the simultaneous use of up to 5 different feeds (pasture, roughage and concentrates), ii) management decisions concerning individual feeds availability can be introduced (this is relevant in systems where the animals receive concentrates only during milking or different feeds at different times during the day), iii) the degradation and passage rates (although they are input as feed characteristics i.e. potential values) are recalculated at each model iteration according to instantaneous substrate availability and rumen fill and iv) it allows the prediction of associative effects between different feeds. The model was evaluated for a wide range of feeding conditions and showed good predictability for total ($R^2 = 0.95$) and forage ($R^2 = 0.92$) DMI, indicating that substitution of forage by concentrate was also predicted close to the observed values. Prediction of NDF digestibility for a smaller range of experimental conditions was poorer ($R^2 = 0.61$). Additionally the model was used to evaluate the effect of level and type of concentrate on DMI (Chilibroste, 1993) and the observed trends agreed with previous research. Despite the satisfactory behaviour of the model to predict DMI for the conditions in which it was evaluated, it has major gaps that should be addressed before a wider applicability is possible. These gaps include the following aspects: i) the model does not apply to grazing conditions, ii) the model only considers rumen fill to be the single constraint to DMI and iii) the model does not predict production and absorption of fermentation end-products, all key process in the problem addressed in the General Introduction.

In Chapter 7 an already existing dynamic simulation model of digestion and absorption of nutrients (Dijkstra et al., 1996) was modified and evaluated under non-steady state conditions. The results of detailed grazing experiments with allowed grazing times and combinations of rumen fill and starvation length before grazing (Chapters 2 and 3) and contrasting sward masses and heights (Chapters 5and 6) as main treatments, were used as reference values. The model was modified to run under conditions of discontinuous feed input of ryegrass. The non-steady state simulation model for rumen fermentation predicted the solid rumen pools (OM, NDF and N) with a favourable root mean square prediction error (MSPE; see Chapter 6 for details) of the observed mean, but still slightly higher than desirable (> 10 %). MSPE was largely inflated by the observations made after the long starvation periods that followed grazing in the grazing experiments. The observed increase in prediction error with increased length of starvation period may be a signal that better definitions of the model inputs are required, mainly fractional passage and degradation rates. Prediction of the soluble fractions

of the OM rumen pool requires also further evaluation. The volatile fractions (VFA and ammonia) showed considerably less satisfactory prediction than the solid fractions. VFA rumen pool was predicted with a root MSPE close to the random variation observed in the experiments. Ammonia rumen pool was poorly predicted and the ammonia representation needs considerable modification to give an accurate prediction of ammonia production and absorption under non-steady state conditions. In its present form the model can be used to predict ruminal digestion and absorption of nutrients (except ammonia) of grazing lactating dairy cows under non-steady state conditions, although it should not be used under conditions that involve long periods of starvation.

Results of the modelling work presented in Chapters 1 and 7 open promising perspectives to shorten the distance between "sward-driven" models (foraging strategies and eventually DMI) and "nutrient-driven" models (digestion and absorption of nutrients). The lack of knowledge about key processes should not hamper model development because modelling may be used to asses quantitatively the consequences of different assumptions. Aggregated models with a sound biological and mathematical representation of relevant processes are valuable predictive tools, although some key aspects remain to be solved empirically for a while. Additionally, models will help to define priorities for research by identifying areas where knowledge is lacking or estimates are too imprecise. Last but not lest, models could be an excellent educational tool and help students to understand the complexity of the systems but still seeing the whole system.

Conclusion and Perspectives

Both conclusions and perspectives have been summarised in one relational diagram presented in Figure 6. The comments that follow are restricted to vegetative temperate swards.

The basic relationships between sward and animal characteristics derived at feeding station level can be extrapolated to plot level (see General Introduction). Hence instantaneous IR can be represented and predicted driven by bite mass as a function of the sward characteristics. Otherwise searching, handling and ingestive behaviour are interspersed at a very small temporal scale in grazing cattle and must be accommodated in model developments. The differentiation between these behaviours is a crucial link between ingestion and digestion. A high IR results in a larger particle size of the DM ingested with direct consequences on rumen volume, digestion and clearance of particles from the rumen and hence on DMI.

Rumen pools can be accurately predicted under discontinuous feeding regimes, although the representation of rumen ammonia pools requires further development. This finding is highly relevant since the distance between the "sward-driven" and "metabolic driven" models can be shortened and the whole and unique process of "ingestion-digestion" of nutrients under grazing tackled.

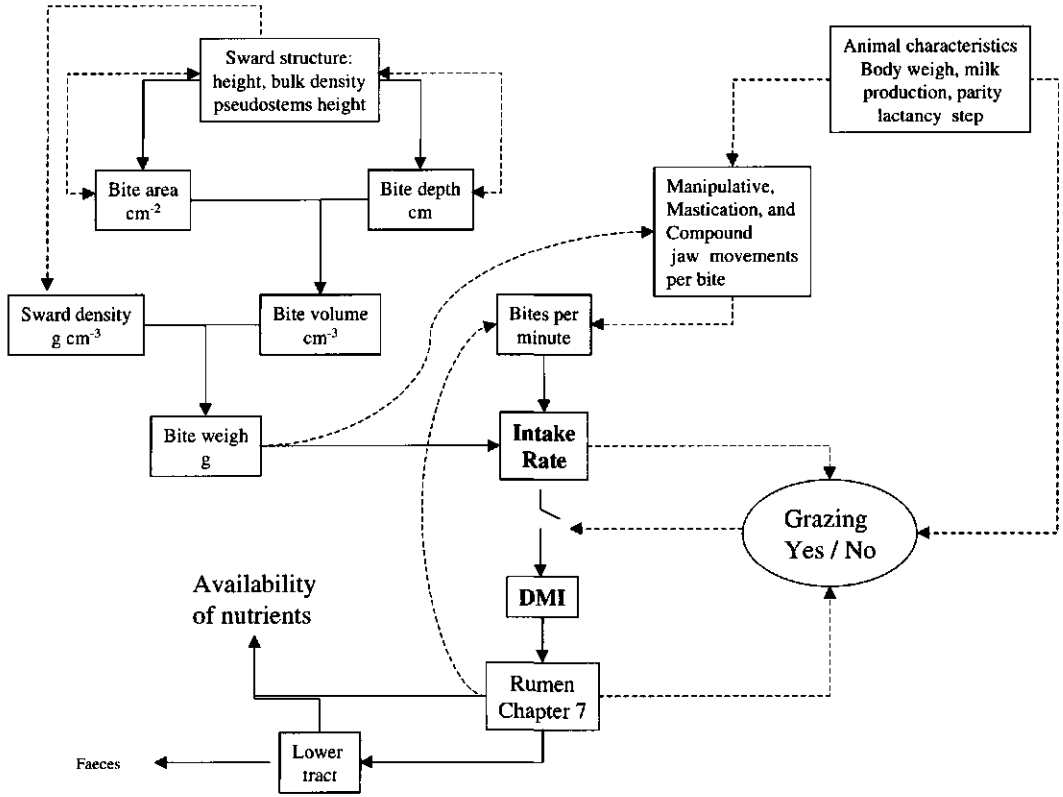


Figure 6: Relational diagram that summarize the main fluxes of materia (solid) and information (dashed), discussed in this Thesis

Grazing time control remains as the more difficult obstacle to understand the whole process. This research offered valuable information about the relative importance of several factors in the control of GT. Clearly it is necessary to understand the way in which the different signals produced at different places are integrated for the animal to modulate eating and other behaviour. In this sense the combination of analytical and synthetic research seems to be an effective strategy.

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SUMMARY

SUMMARY

Grazing systems rely on the direct utilisation of the produced grass and its efficiency is the main issue by which productivity and profitability of these systems in market-oriented countries is determined. The problems of grass production and utilisation may be looked upon from a whole paddock, animal or plant morphology perspective. In assessing grass production and utilisation from an animal perspective, dry matter intake (DMI) is the dominant process. In intensively managed grazing systems DMI is the most appropriate criterion on which to base tactical or within season management decisions including:

- level and type of supplementation for the dairy herd,
- grazing area allocation,
- grazing system,
- control of grazing time and daily schedule,
- grouping of cows, etc.

Daily DMI may be seen as the summation of individual discrete meals. Under grazing conditions, grazing behaviour is characterised by alternating periods of eating and periods of fasting. For lactating dairy cows, two major grazing bouts have been observed: one in the morning and the largest in the afternoon. Under daily strip-grazing, this ingestive behaviour may be even more pronounced with a quick depletion of the available pasture. Understanding which factors control a meal would lead to the understanding of daily DMI although it is recognised that different factors may operate at different times during the day. On a daily basis, DMI under grazing is commonly expressed as the product of intake rate (IR) and grazing time (GT). In recent decades substantial progress has been made in the understanding and quantification of the main mechanisms determining IR. The bite has been recognised as the functional link between the animal and the sward and the connection between the individual bite and IR can be defined arithmetically: IR over a given time period is the product of the bite mass and mean bite rate over that period. In contrast to the significant progress that has been made on IR, control of GT still remains poorly understood.

The interactions of animal and sward variables create a complex system and from this perspective an interest in modelling is not only justified, but it is most likely the only way to accommodate the complexity of the system. A proper balance between modelling and analytical research has been proven an effective strategy to improve both understanding and quantification of complex systems. In the last decades significant progress has been made in modelling foraging strategies, forage quality, and whole rumen function, but, unfortunately almost without making the crucial connection between the different fields. For the foraging models the digestion and absorption of nutrients has frequently been ignored and in the rumen function models DMI is considered a defined and often continuos input.

In this thesis analytical (grazing and *in-vitro* trials) and synthetic (modelling) approaches were combined with the following objectives:

1. To gain insight in the main mechanisms controlling DMI, IR and GT during the first grazing session after a.m. milking. Effects of the length of grazing session, the combination of starvation and rumen fill before grazing, and the sward characteristics on

ingestive behaviour (bite mass and bite rate), IR and GT were studied (Chapters 2, 3, 5 and 6).

2. To judge the relative importance of rumen fill and the concentration of fermentation products in the rumen liquor as candidates to signal the end of the grazing sessions (Chapters 2, 3, 5 and 6).

3. To evaluate the use of the gas production technique as an alternative *in-vitro* method to characterise rumen content fermentability in animals exposed to different treatments involving periods of starvation, rumen fill and allowed grazing time (Chapter 4).

4. To develop (Chapter 1) and design, modify and evaluate (Chapter 7) an existing simulation model to operate under non-steady state conditions with the aim either to predict DMI (Chapter 1) or to predict rumen fermentation and supply of nutrients (Chapter 7).

Grazing experiments

Chapters 2 and 3. The effects of the length of allowed grazing time (Experiment 1), and length of a starvation period before grazing (Experiment 2) on DMI, ingestive behaviour, liquid and particulate rumen pool sizes, composition and fermentability, during the first grazing bout were investigated. For that purpose rumen content was evacuated, weighed, sampled and returned to the animals at different times. In Experiment 1 four lengths of allowed grazing time (1, 1.75, 2.50 and 3.25 h) after overnight starvation were compared. Increasing the length of the allowed grazing time significantly increased DMI ($P<0.01$), the proportion of time spent actively eating ($P<0.01$) and DM rumen pool size after grazing ($P<0.05$). However, DM rumen pool size after grazing was smaller than that measured immediately before the start of the fasting period ($P<0.01$). Bite mass during the first hour of grazing was greater than in the following grazing time. In both experiments bite rate declined as the grazing session progressed, but bite rate was not affected by treatments. The allowed grazing time did not have any significant effect on total and liquid rumen pool sizes after grazing but did have ($P<0.05$) on DM and OM (slope, 0.5 kg h^{-1}) rumen pool sizes. The non-significant differences between the volatile fatty acids (VFA) rumen pool sizes before and after 1 hour of grazing indicate a delay in the availability for the microorganisms of the more rapidly fermentable substrate. The total VFA rumen pool sizes increased significantly ($P<0.01$; slope, 1.88 mol h^{-1}) with the allowed grazing time, which suggests that these fermentation products may be involved in the control of the grazing time in later stages during the day. Experiment 2 consisted of a factorial combination of two durations of starvation before grazing (16.5 [LS] and 2.5 [SS], h) and the presence or absence in the rumen of 12.5 kg of a synthetic indigestible material. DMI as well as GT were greater after a starvation period of 16.5 h and were reduced by the presence in the rumen of indigestible material ($P<0.01$). The interaction between factors on GT, although not significant ($P<0.06$), supports the idea of a combination of signals controlling meal size under grazing conditions. Bite mass was not significantly modified by period of starvation. The duration of starvation before grazing did not affect significantly the particulate, ammonia and VFA rumen pool sizes after grazing except for propionic acid which was reduced ($P<0.05$) by the larger starvation time. The inclusion of inert bulk material in the rumen before grazing significantly reduced

($P<0.05$) the total, liquid, DM, OM and ammonia rumen pool sizes but not the VFA rumen pool sizes after grazing. High levels of ammonia as well as total rumen contents may be involved in the control of the grazing time in this experiment.

Chapters 5 and 6. The relative importance of duration of sward regrowth and rumen content characteristics and fermentation, on the control of grazing time during the first grazing session of the day were studied. Four lactating dairy cows were allowed to graze ryegrass (*Lolium Perenne*) swards, which had been allowed 5 different regrowth periods after mowing (6, 9, 16, 22 and 30 d). The cows were allowed to graze until they stopped voluntarily. Before and after grazing the rumen content was evacuated, weighed, sampled and returned to the animals. Samples of rumen liquid were taken immediately before rumen evacuation, and approximately 30, 60, 120 and 240 minutes after the grazing session was finished. Grazing time did not follow a significant trend with period of regrowth, and exhibited a profound discontinuity between experimental days 6 and 16. When the pasture had 9 days of regrowth the cows attempted to compensate for a reduced bite mass and intake rate by increasing grazing time. Neither intake rate nor bite mass exhibited a significant trend with period of regrowth. The lowest and highest values of intake rate and bite mass were observed on regrowth days 9 and 30 respectively. Bite rate did not change significantly with duration of regrowth with cows exhibiting high rates of biting for all the sward conditions. Both the total and DM rumen pools sizes after grazing increased significantly with the days of regrowth ($P < 0.01$). Nevertheless the cows always stopped grazing before a maximum rumen capacity was reached. Rumen pool sizes of OM and NDF measured after grazing increased significantly ($P < 0.05$) with sward days of regrowth even though the absolute values were low. Volatile fatty acid rumen pools size increased linearly ($P <0.05$) with age of regrowth. Concentration of VFA followed a significant quadratic trend with a maximum concentration observed at approximately 110 min after cessation of grazing. Nitrogen fractions (N, ammonia) did not follow any significant trend with days of regrowth. In this study, rumen fill (as represented by total, DM or NDF rumen pools size), VFA (either total or major components), ammonia, pH and osmotic pressure as individual variables were not correlated with grazing time or DMI. It would appear that, rather than a single absolute level of any of these variables being responsible, a combination of these signals may control the initiation and termination of individual meals.

In-vitro experiments

Chapter 4. Fermentability of rumen content samples, collected during the grazing trials reported in Chapters 2 and 3, were investigated by means of measuring their cumulative gas production after incubation with an inoculum of sheep rumen fluid. Rumen samples taken after 1 hour of grazing in Experiment 1 had higher values of total accumulated gas with less ($p<0.05$) time required to reach the maximum fermentation rate than cows grazed for 3.25 hours. In Experiment 2 after the grazing session, the total gas of rumen samples from the LS cows were significantly higher ($P<0.05$) than for the SS cows. This was in agreement with the observed higher DMI during grazing and DM rumen pools after grazing in LS cows. For both starvation periods, the presence of inert rumen bulk led to a higher total gas, a shorter half time and less DM left unfermented. Before grazing the total accumulated gas production was less ($P<0.05$) for the LS than for the SS cows. The measurement of fermentation kinetics by cumulative gas production was suitable to detect changes in rumen content fermentation

patterns due to the clearance of material from the rumen (effect of starvation) or DMI during the grazing sessions.

Modelling

Chapter 1. A stochastic, dynamic simulation model, aimed to predict DMI under discontinuous feeding regimes with a combination of up to 5 different feeds, was developed. The model was evaluated for a wide range of feeding conditions and showed good predictability for total (R^2 = 0.95) and forage (R^2 = 0.92) DMI, indicating that substitution of forage by concentrates was also predicted close to the observed values. Prediction of NDF digestibility for a smaller range of experimental conditions was poorer (R^2 = 0.61). Additionally the model was used to evaluate the effect of level and type of concentrate on DMI and the observed trends agreed with previous research results. Despite the satisfactory behaviour of the model to predict DMI for the conditions in which it was evaluated, two important limitation remains for a wider applicability: the model only considers rumen fill to be the single constraint to DMI and the model does not predict production and absorption of fermentation end-products.

Chapter 7. A dynamic simulation model of digestion and absorption of nutrients (Dijkstra et al., 1996) was redesigned, modified and evaluated under non-steady state conditions. The results of the grazing experiments (Chapters 2, 3, 5 and 6) were used as reference values. The model was modified to run under a discontinuous feed input of ryegrass. Neutral Detergent Fibre (NDF) and nitrogen (N) rumen pools were predicted with a relatively low root mean square prediction error (MSPE) of the observed means (12 %) for experiments 1 and 2 but higher values (18.1 %) were observed for experiment 3. The root MSPE was significantly inflated by the long period of starvation (length of starvation period up to 20.5 hours) that followed grazing in the three experiments. Organic matter (OM) rumen pool was poorer predicted (root MSPE of 16 %) than NDF and N rumen pools and requires further validation. Volatile fatty acids (VFA) rumen pool and VFA concentration were predicted with a root MSPE of the observed mean of 32 to 33 % which was close to the random variation observed in the experiments. Ammonia rumen pool was poorly predicted and the way ammonia is represented in the model requires severe modification to predict ammonia production and absorption under non-steady state conditions. It was concluded that the model can be used under discontinuous feeding regimens to predict ruminal digestion and absorption of nutrients (except ammonia) in grazing lactating dairy cows, although feeding regimens that would involve large periods of starvation must be avoided.

Conclusions

In the General Discussion the results from the grazing and *in-vitro* experiments were analysed as a pooled data set and put in perspective. A conceptual model comprising the highly relevant fluxes of material and information, discussed in this research but also required for further developments, was drawn. The basic relationships between sward and animal characteristics derived at feeding station level can be extrapolated to plot level Hence instantaneous IR can be represented and predicted as driven by bite mass as a function of the sward characteristics. Otherwise searching, handling and ingestive behaviours are interspersed

at a very small temporal scale in grazing cattle and must be accommodated in model developments. The differentiation between these behaviours is a crucial link between ingestion and digestion. A high IR results in a larger particle size of the DM ingested with direct consequences on rumen volume, digestion and clearance of particles from the rumen and hence on DMI. Rumen pools can be accurately predicted under discontinuous feeding regimes, although the representation of rumen ammonia pools requires further development. This general finding is highly relevant since the distance between the "sward-driven" and "metabolic driven" models can be shortened and the whole and unique process of "ingestion-digestion" of nutrients under grazing tackled. Grazing time control remains a difficult obstacle to understand the whole process. This research offered valuable information about the relative importance of several factors in the control of GT. Clearly it is necessary to understand the way in which the different signals produced at different places are integrated for the animal to modulate eating and other behaviour. In this sense the combination of analytical and synthetic research was proven to be an effective strategy.

SAMENVATTING

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Op gras en begrazing gebaseerde systemen van dierlijke productie zijn afhankelijk van de directe benutting van het staande en nog groeiende gewas. In landen die afhankelijk zijn van de wereldmarkt is deze benutting een belangrijk gegeven dat bepalend is voor hun productiviteit en economisch rendement. De productie en benutting van gras kan beschouwd worden vanuit verschillende gezichtspunten, nl. vanuit het perspectief van het begraasde oppervlak, vanuit het dier of vanuit de platenmorfologie. Bij grasbenutting vanuit het dier gezien is de opname aan drogestof (DMI) van overwegend belang. In intensieve begrazingssystemen is DMI het meest geschikte criterium op grond waarvan de door het groeiseizoen gedicteerde of andere tactische beslissingen genomen kunnen worden. Tot deze beslissingen behoren:

- de hoogte en de soort van bijvoedering voor de melkveestapel ,
- het aangeboden graasoppervlak ,
- het begrazingssysteem,
- de controle over de graastijd en het dagelijkse schema,
- de groepsindeling van koeien, enz. .

De DMI per dag kan beschouwd worden als de som van de individuele discrete maaltijden. Onder begrazing wisselen vreetperiodes en periodes van vasten elkaar af. Voor melkgevende koeien zijn twee grote graasperiode bekend: één in de ochtend en de grootste in de middag of vroege avond. Bij het toepassen van dagelijkse strip begrazing wordt het opnamegedrag nog belangrijker omdat het beschikbare gras dan heel snel uitgeput raakt. Een beter begrip van welke factoren het begin en het einde van een maaltijd beinvloeden zou ook het inzicht vergroten in de DMI per dag, waarbij wel in het oog gehouden moet worden dat verschillende factoren op verschillende tijdstippen gedurende de dag van meer of minder belang kunnen zijn. Op dagbasis wordt de DMI onder begrazing doorgaans uitgedrukt als het product van opnamesnelheid (IR) en graastijd (GT). Gedurende de afgelopen decennia is grote voortgang geboekt in het begrijpen en kwantificeren van de belangrijkste mechnismen die de opnamesnelheid bepalen. De hap is herkend als de functionele verbinding tussen het dier en het gewas. De verbinding tussen de individuele hap en de opname snelheid kan rekenkundig worden weergegeven: de opnamesnelheid over een bepale tijdsperiode is het product van de hapgrootte en de gemiddelde vreetsnelheid (happen/minuut) over die peride. In tegenstelling tot de grote vooruitgang die is geboekt op het gebied van de opnamesnelheid bestaat er nog slechts weinig inzicht in de factoren die van beslissende invloed zijn op de graastijd

De interactie tussen dierfactoren en gewasfactoren vormt een complex systeem en vanuit dit gezichtspunt is het modelmatig weergeven ervan niet alleen verantwoord, maar wellicht zelfs de enige manier om de complexiteit van het systeem te hanteren en te ontrafelen. Een evenwichtige balans tussen modelmatige weergave en dierexperimenteel onderzoek is een effectieve strategie voor het verbeteren van ons inzicht en het op kwantitatieve wijze weergeven van dit complexe systeem. Gedurende de laatste decennia is er grote vooruitgang geboekt op het gebied van het modelmatig weergeven van begrazingsstrategieën, graskwaliteit en pensfermentatie, maar het leggen van een (modelmatig) verband tussen de verschillende onderdelen is helaas vaak achterwege gebleven. In begrazingsmodellen zijn vertering en de absorptie van nutriënten vaak veronachtzaamd en in het model waarin

pensfermentatie wordt weergegeven wordt DMI als gegeven beschouwd en vaak gehanteerd als een constante input van het model.

In het in dit proefschrift beschreven onderzoek worden de dierexperimentele analytische (begrazing en *in vitro* onderzoek) en de synthetiserende (modelmatige) benadering gecombineeerd met de navolgende doelstellingen:

5. Inzicht te krijgen in de belangrijkste mechanismen die ruwvoeropname (DMI), opnamesnelheid (IR) en graastijd (GT) gedurende de eerste graasperiode na het ochtendmelken beinvloeden. Effecten die bestudeerd zijn waren de lengte van de graastijd, de combinatie van vasten en pensvulling voorafgaande aan grazen en de eigenschappen van het staand gewas op opnamegedrag (hapgrootte en vreetsnelheid), opnamesnelheid en graastijd (hoofdstukken 2, 3, 5 en 6).

6. Het beoordelen van het relatieve belang van pensvulling en de concentratie van eindproducten van fermentatie in de pensvloeistof als mogelijke kandidaten voor het signaal dat het einde van een graastijd aangeven (hoofdstukken 2, 3, 5 en 6).

7. Het evalueren van het gebruik van de gasproductie test als een alternatieve *in vitro* methode voor het vaststellen van de fermenteerbaarheid van de pensinhoud in koeien die waren blootgesteld aan verschillende behandelingen, waaronder perioden van vasten, (kunstmatige) pensvulling en toegelaten graastijd (hoofdstuk 4).

8. Het ontwikkelen (hoofdstuk 1) en het ontwerpen, aanpassen en evalueren (hoofdstuk 7) van simulatiemodellen die werken onder niet steady-state omstandigheden, met het doel hetzij de voeropname (DMI), hetzij pensfermentatie en nutriëntenaanbod te voorspellen.

Begrazingsproeven

Hoofdstukken 2 en 3. De effecten van de tijdsduur van de toegelaten graastijd (proef 1), en de tijdsduur van een periode van vasten voorafgaande aan de graastijd (proef 2) op voeropname, opnamegedrag, omvang van de poolgroottes in de pens van vloeibare en vaste bestanddelen, en samenstelling en fermenteerbaarheid van pensinhoud tijdens de eerste graasperiode na het melken werden onderzocht. Voor dat doel werd op verschillende tijdstippen de volledige pensinhoud geëvacueerd en na te zijn gewogen en bemonsterd in het dier teruggeplaatst. In proef 1 werden de effecten van 4 verschillende toegelaten graastijden (1; 1,75; 2,50 en 3,25 uur) na een periode van vasten gedurende de voorafgaande nacht, vergeleken. Het toelaten van een langere graasperiode veroorzaakte een significante (P<0,01) toename van DMI, van het deel van de tijd die besteed werd aan actief vreten (P<0,01) en van de poolgrootte van de drogestof (DM) in de pens na grazen (P<0,05). De poolgroote van DM in de pens bleef echter kleiner dan die welke gemeten werd vlak voordat de periode van vasten begon.(P<0,01). De happen gedurende het eerste uur grazen waren groter in omvang dan in de daaropvolgende graastijd. In beide proeven nam de hapgrootte af met het toenemen van de lengte van de graasduur, maar de vreetsnelheid (happen/minuut) werd niet beinvloed door de lengte van de toegelaten graastijd. De lengte van de toegelaten graastijd had geen significante invloed op de poolgroottes van de totale pensinhoud of pensvloeistof aan het einde van de graasperiode maar wel (P<0,05) op de poolgroottes van DM en OM die met 0,5

kg h^{-1} in omvang toenamen. De niet-significante verschillen in de poolgroottes van de vluchtige vetzuren (VFA) onmiddellijk voorafgaande aan en na een graastijd van 1 uur zijn een indicatie voor een vertraagde beschikbaarheid van snel fermenteerbaar substraat voor de pensmicroben. Met het toenemen van de lengte van de toegestane graastijd nam de poolgrootte van de totale VFA significant (P<0.01; toename, 1.88 mol h^{-1}) in omvang toe, wat een aanwijzing is dat deze eindproducten van fermentatie een rol spelen in de controle van graasgedrag en graastijd in de latere dagdelen. Proef 2 bestond uit een factoriële combinatie van 2 tijdsduren van vasten voorafgaand aan grazen (16,5 [LS] and 2,5 [SS], h) en de aan- of afwezigheid in de pens van 12,5 kg van een onverteerbaar synthetisch materiaal. Zowel de opname aan drogestof (DMI) als de graastijd (GT) waren groter na een periode van vasten van 16,5 uur en waren kleiner bij de aanwezigheid in de pens van onverteerbaar materiaal (P<0,01). De aanwezigheid van een interactie tussen de invloed van de behandelingfactoren op GT, hoewel niet significant (P<0,06), ondersteunt de opvatting dat bij begrazing de maaltijdgrootte geregeld wordt door een combinatie van signalen. De hapgrootte werd niet significant veranderd door de lengte van de periode van vasten. De lengte van de periode van vasten voorafgaand aan het grazen had geen significante invloed op de poolgroottes in de pens na het grazen van vaste deeltjes, ammoniak en VFA, uitgezonderd die van propionzuur welke kleiner was (P<0,05) na een langere periode van vasten. Het plaatsen en de aanwezigheid van inert bulk materiaal in de pens voorafgaand aan het grazen verkleinde de omvang van de pools van totale pensinhoud, pensvloeistof, DM, OM en ammoniak significant (P<0,05) na grazen, maar had geen invloed op de pool van totaal VFA. Zowel hoge gehaltes aan ammoniak als totale pensinhoud kunnen een rol gespeeld hebben bij de controle van de graastijd in deze proef.

Hoofdstukken 5 en 6. In deze proeven werd het relatieve belang onderzocht van de lengte van de periode van hergroei van het gewas en eigenschappen van pensinhoud en pensfermentatie op de controle van graastijd tijdens de eerste graasperiode na het ochtendmelken. Voor dat doel werd aan 4 melkgevende koeien toegestaan te grazen op veldjes raaigras (*Lolium Perenne*), die na maaien gedurende 5 verschillende perioden van hergroei (6, 9, 16, 22 en 30 d) hadden ondergaan. De koeien mochten grazen tot zij daarmee uit eigener beweging stopten. Vlak voor en na het stoppen van grazen werd de volledige pensinhoud geëvacueerd en, na te zijn gewogen en bemonsterd, in het dier teruggeplaatst. Van de pensvloeistof werden onmiddellijk voor de pensevacuatie en ongeveer 30, 60, 120 en 240 minuten na het beëindigen van de graasperiode monsters genomen. Er werd geen significant verband gevonden tussen de lengte van de (vrijwillige) graastijd en de lengte van de periode van hergoei. Met name de dagen 6 en 16 lieten een grote discrepantie zien. Toen de grasveldjes een hergroeiperiode van 9 dagen achter de rug hadden probeerden de koeien kennelijk om een sterk verlaagde hapgrootte en opname te compenseren door de graastijd te verlengen. Noch de snelheid van opname noch de hapgrootte vertoonden een aan de periode van hergroei gerelateerde trend. De laagste en hoogste waarden voor opnamesnelheid en hapgrootte werden waargenomen op respectievelijk de hergroeidagen 9 en 30. Vreetsnelheid werd niet significant beinvloed door de lengte van de periode van hergroei. Onder alle condities van hergroei werden hoge vreetsnelheden waargenomen. Zowel de poolgroottes van de totale pensinhoud als van de DM namen significant (P<0,01) in omvang toe met een toenemende lengte van de periode van hergroei. Ondanks dat stopten de koeien altijd met grazen voordat hun maximale capaciteit van pensvulling was bereikt. De na afloop van grazen gemeten poolgroottes in de pens van OM en NDF namen significant (P < 0.05) in omvang toe met een toename van het aantal dagen hergroei, maar hun absolute omvang bleef laag. De

omvang van de poolgroottes van VFA in de pens namen lineair toe met het toenemen van het aantal dagen hergroei. De VFA concentraties vertoonden een significant kwadratische toename met de tijd, met een maximale concentratie op ongeveer 110 minuten na het stoppen met grazen. Stikstof (N) fracties (N, ammoniak) lieten geen enkele trend van betekenis zien met een toename van het aantal dagen hergroei. In deze proef werd geen verband gevonden tussen pensvulling (weergegeven door de poolgrootte van totale pensinhoud, DM of NDF), VFA (totaal of de individuele zuren), ammoniak, pH en osmotische druk met graastijd of DMI. Het lijkt er op dat eerder een combinatie van al deze signalen dan een individueel signaal verantwoordelijk is voor het controleren van het initiëren of beëindigen van individuele maaltijden.

In-vitro experimenten

Hoofdstuk 4. De monsters pensinhoud, verzameld tijdens de in hoofdstukken 2 en 3 gerapporteerde graasproeven, werden onderzocht op hun fermenteerbaarheid door middel van het meten van hun cumulatieve gasproductie tijdens *in vitro* incubatie met een inoculum van pensvloeistof. Monsters pensinhoud, verzameld in proef 1 na 1 uur grazen, hadden ten opzichte van de monsters verzameld na 3,5 uur grazen, een hogere totale gasproductie, en een kortere tijd ($P<0,05$) nodig om de maximale fermentiesnelheid te bereiken. In proef 2 resulteerden de monsters pensinhoud afkomstig van de LS koeien in een significant hogere ($P<0,05$) totale gasproductie. Dit kwam overeen met de tijdens dezelfde graasperiode waargenomen hogere DMI en de hogere poolgroottes aan DM in de LS koeien. In beide periodes van vasten leidde de aanwezigheid van inert bulk materiaal tot een hogere totale gasproductie, een kortere halfwaardetijd en een lager aandeel van de DM dat niet werd gefermenteerd. In vergelijking met de SS koeien resulteerden de voor het begin van de graasperiode bij de LS koeien genomen monsters in een significant ($P<0,05$) lagere gasproductie. De met cumulatieve gasproductie bepaalde fermentatie kinetiek bleek in staat verschillen in pensfermentatiepatronen als gevolg van verschillen in pensvulling na vasten en DMI tijdens de daaropvolgende graasperiode aan te tonen.

Modellering

Hoofdstuk 1. Een stochastisch, dynamisch computersimulatie model, dat als doel had de DMI onder discontinue voedering te voorspellen werd ontwikkeld. Het model kent een combinatie van maximaal 5 verschillende voeders. Het model werd geëvalueerd voor een brede reeks van voedingsomstandigheden en bleek zowel de totale ($R^2=0.95$) als de ruwvoer ($R^2=0.92$) DMI goed te voorspellen, wat sterke aanwijzingen zijn dat de verdringing van ruwvoer door krachtvoer ook met een hoge mate van nauwkeurigheid werd voorspeld. De voorspelling van de NDF vertering voor een betrekkelijk klein aantal rantsoenomstandigheden was minder nauwkeurig ($R^2=0.61$). Het model werd ook gebruikt om het effect van hoeveelheid en type krachtvoer op DMI te voorspellen en de voorspelde trends kwamen goed overeen met de resultaten van eerder onderzoek. Ondanks dat het model zich bevredigend gedroeg voor wat betreft het voorspellen van de DMI onder de omstandigheden waaronder het werd geëvalueerd, staan twee belangrijke tekortkomingen een bredere toepasbaarheid in de weg: het model beschouwt pensvulling als de enige beperking

voor DMI en het model doet geen voorspelling over de productie en absorptie van de eindproducten van pensfermentatie.

Hoofdstuk 7. Een reeds bestaand dynamisch simulatie model dat de vertering en absorptie van nutriënten voorspelt (Dijkstra et al., 1996), werd aangepast en geëvalueerd onder niet-steady state omstandigheden. Hiervoor werden de in de hoofdstukken 2, 3, 5 en 6 vermelde resultaten als referentiewaarden gebruikt. Het model werd zodanig uitgebreid en aangepast dat een discontinue input van raaigras als input kon worden gebruikt. Het model voorspelde de waargenomen gemiddelde poolgroottes van Neutral Detergent Fibre (NDF) and stikstof (N) in de pens vrij nauwkeurig en met een relatief lage (12%) "root mean square prediction error" (MSPE) voor de proeven 1 en 2; voor proef 3 werden echter hogere MSPE waarden (18.1%) gevonden. Het bleek dat de MSPE waarden sterk negatief werden beïnvloed door de lange periode van vasten (tot maximaal 20,5 uur) die volgde op de graasperiode in de drie proeven. De voorspelnauwkeurigheid van de poolgrootte van OM in de pens was lager (MSPE:16%) dan die van NDF en N. De reden hiervan vereist nader onderzoek en validatie. Zowel de poolgroottes van VFA als de VFA concentraties in de pens werden voorspeld met een MSPE van 32-33% van de waargenomen gemiddeldes, wat in de buurt ligt van de in de proeven waargenomen "random" variatie. De omvang van de poolgrootte van ammoniak in de pens werd door het model slecht voorspeld. Hieruit moet worden geconcludeerd dat de wijze waarop het gedrag van ammoniak in het huidige model wordt weergegeven, aanzienlijke aanpassing behoeft om de productie en absorptie van ammoniak onder niet-steady state omstandigheden bevredigend te voorspellen. Geconcludeerd kan worden dat het model bruikbaar is om de pensvertering en nutriëntenabsorptie bij grazende lacterende koeien onder discontinue voerstrategieën te voorspellen. Een voorbehoud wordt gemaakt voor het voorspellen van het gedrag van ammoniak en voor situaties waarbij lange perioden van vasten een rol spelen.

Conclusies

In de General Discussion werden de resultaten van de begrazings- en *in-vitro* proeven geanalyseerd als een gepoolde data set en vervolgens in het juiste perspectief geplaatst. Een conceptueel model waarin de meest relevante "fluxen" van materiaal en informatie een rol spelen, werd ontwikkeld en besproken. Ook worden onderzoekgebieden aangegeven die nader onderzoek vragen. De basale relaties tussen de eigenschappen van een staand gewas en het dier zoals vastgesteld op het niveau van een een graspol simulerend "voerstation" kunnen worden geëxtrapoleerd naar het niveau van het proefveld. Met andere woorden: de op zeker moment waargenomen IR kan worden weergegeven en voorspeld als aangedreven door hapgrootte als functie van de gewasstructuur. Verder zijn bij grazende koeien het gedrag m.b.t. het zoeken, hanteren en opname van voedsel met elkaar verweven in en op een zeer kleine tijdschaal, waarmee in het ontwikkelen van modellen rekening moet worden gehouden. Het onderscheid tussen deze gedragskenmerken is een cruciale schakel tussen opname en vertering. Een hoge IR geeft een grotere deeltjesgrootte van de opgenomen DM met directe consequenties voor pensvolume, pensvertering en de verwijdering van voerdeeltjes uit de pens en dus voor DMI. Poolgroottes in de pens kunnen redelijk nauwkeurig worden voorspeld onder discontinue voerstrategieën, maar de weergave van de ammoniak pools in de pens vereist verdere ontwikkeling. Deze waarneming heeft grote relevantie omdat hiermee de afstand tussen "sward-driven" en "metabolic driven" modellen verkleind kan worden en ook

het hele proces van opname en benutting van nutriënten bij begrazing aangepakt kan worden. De controle van de graastijd blijft een moeilijk obstakel bij het proberen te begrijpen van het hele proces. Dit onderzoek heeft waardevolle informatie opgeleverd over het relatieve belang van verscheidene factoren die een rol spelen in de controle van GT. De resultaten onderstrepen ook de noodzaak van het eerst moeten begrijpen op welke wijze signalen die op verschillende plaatsen ontstaan voor en door het dier geïntegreerd worden, om vervolgens het vreet- en ander gedrag te moduleren. In dat opzicht blijkt de combinatie van "analytisch" en "synthetisch" onderzoek een effectieve strategie.

RESUMEN

RESUMEN

Los sistemas pastoriles se basan en la utilización directa del forraje producido y la eficiencia de producción y utilización del forraje producido, son determinante de la productividad y rentabilidad en sistemas de producción no subsidiados. La producción y utilización del forraje puede ser analizadas desde la perspectiva de la pastura, del animal o de la morfología de las plantas. Desde la perspectiva animal, el consumo de materia seca (CMS) es el proceso dominante. Adicionalmente, el CMS es el criterio más apropiado en sistemas pastoriles intensivos, sobre el cual basar decisiones de tipo táctico o de manejo tales como:

- nivel y tipo de suplemento para el rodeo,
- asignación de área,
- sistema de pastoreo,
- control del tiempo de pastoreo,
- formación de grupos de alimentación, etc.

El CMS diario puede ser visualizado como la sumatoria de comidas discretas individuales. En condiciones de pastoreo los animales normalmente alternan períodos de consumo de forraje con períodos de ayuno. En vacas lecheras, se han observado dos sesiones principales de pastoreo: una en la mañana y la más larga en la tarde. Cuando se realiza pastoreo en franjas diarias, ese comportamiento puede ser aún más pronunciado con una rápida desaparición del forraje disponible. La comprensión de los factores que controlan el inicio y fin de las sesiones individuales de pastoreo, permitirá la comprensión del CMS diario, a pesar de que distintos factores, pueden estar involucrados en el inicio y fin de la sesión de pastoreo en distintos momentos del día. En base diaria, el CMS en pastoreo es comúnmente expresado como el producto de la tasa de consumo (TC) por el tiempo de pastoreo (TP). En las últimas décadas, se ha registrado un progreso substancial en la comprensión y cuantificación de los mecanismos que determinan la TC. A nivel del bocado individual ha sido reconocido el vínculo funcional entre el animal y la pastura. La relación cuantitativa entre bocados individuales y TC puede ser definida aritméticamente: TC en un período de tiempo acotado, es el producto de la tasa y el peso de bocado, en dicho período. En contraste al importante progreso observado en la identificación y cuantificación de los mecanismos que regulan la TC instantánea, el control del TP permanece aún pobremente comprendido.

Las fuertes interacciones entre variables del animal y de la pastura, crean un sistema complejo y desde ésta perspectiva, el interés en el análisis sistémico no es sólo justificado, sino tal vez la única vía para integrar la complejidad del sistema bajo estudio. Un balance apropiado entre técnicas analíticas y sintéticas ha probado ser una estrategia efectiva para mejorar tanto la comprensión como la cuantificación de sistemas complejos. En las últimas décadas, se han logrado progresos significativos en la modelación del proceso de pastoreo, variaciones en calidad del forraje y funcionamiento del rumen, pero desafortunadamente, sin explorar aún el vínculo crucial entre éstos campos del conocimiento. En los modelos de comportamiento ingestivo la digestión y absorción de nutrientes ha sido frecuentemente ignorada y en los modelos de rumen el CMS es normalmente considerado como una variable de entrada y simulando condiciones continuas de alimentación.

En esta Tesis se utilizaron aproximaciones analíticas (experimentos en pastoreo e *in-vitro*) y sintéticas (modelos de simulación), con los siguientes objetivos:

1. Comprender los principales mecanismos de control del CMS, TC y TP durante la primer sesión de pastoreo después del ordeñe a.m. Con tal propósito se estudiaron los efectos del largo de la sesión de pastoreo, la combinación de diferentes tiempos de ayuno y niveles de llenado ruminal previo a la sesión de pastoreo y las características de la pastura, sobre el TP y la tasa y peso de bocado (**Capítulos 2, 3, 5 y 6**).
2. Juzgar la importancia relativa del llenado y concentración de productos de la fermentación en el rumen, como candidatos responsables de la señalización del fin de la sesión de pastoreo (**Capítulos 2, 3, 5 y 6**).
3. Evaluar el uso de la técnica de producción de gas, como un método *in-vitro* alternativo, para caracterizar la fermentabilidad del contenido ruminal de vacas en producción, expuestas a diferentes tratamientos, involucrando períodos de ayuno y llenado artificial del rumen previo al pastoreo, así como diferentes tiempos de acceso a la pastura (**Capítulo 4**).
4. Desarrollar (Capítulo 1) y diseñar, modificar y evaluar un modelo existente de rumen (Capítulo 7), con el objetivo de predecir el CMS (**Capítulo 1**) y la fermentación en el rumen y suministro de nutrientes (**Capítulo 7**), en condiciones discontinuas de alimentación.

Experimentos en pastoreo

Capítulos 2 y 3. El efecto del largo de la sesión de pastoreo (Experimento 1), y el largo del período de ayuno previo al pastoreo (Experimento 2) sobre el CMS, comportamiento ingestivo, contenido, composición y fermentabilidad del rumen, durante la primer sesión de pastoreo después del ordeñe a.m., fueron investigadas. Con éste propósito, el contenido ruminal fue evacuado, pesado, muestreado y retornado a los animales en diferentes momentos durante el día. En el Experimento 1, los animales tuvieron acceso a la pastura durante 1, 1.75, 2.5 y 3.25 horas. La noche anterior a la sesión de pastoreo los animales fueron ayunados por aproximadamente 16 h. Los incrementos en el tiempo permitido de acceso a la pastura, aumentaron significativamente el CMS ($P<0.01$), la proporción de tiempo en que los animales estuvieron efectivamente pastoreando ($P<0.01$) y la cantidad de contenido total del rumen, posterior al pastoreo ($P<0.05$). Sin embargo, la cantidad de MS en el rumen al final de la sesión de pastoreo, fue menor que la medida inmediatamente antes de comenzar el período de ayuno ($P<0.01$). El peso de bocado durante la primer hora de pastoreo, fue mayor que en las horas siguientes. En ambos experimentos, la tasa de bocado disminuyó con el tiempo de pastoreo, aunque no fue significativamente afectada por los tratamientos. El largo de la sesión de pastoreo no tuvo efectos significativos en la cantidad total de contenido ruminal, aunque sí afecto significativamente ($P<0.05$) la cantidad de MS y MO del rumen (pendiente, 0.5 kg h^{-1}). No se detectaron diferencias significativas en el pool de ácidos grasos volátiles (AGV) previo y posterior a 1 hora de pastoreo, lo que sugiere una demora en la liberación del substrato más rápidamente fermentable. El pool de AGV se incrementó significativamente ($P<0.01$, pendiente, 1.88 moles/h) con el tiempo de pastoreo, lo que sugiere que los productos finales de la

fermentación podrían estar involucrados en el control del cese de la actividad de pastoreo, en etapas posteriores del día. El Experimento 2, consistió en una combinación factorial de dos tiempos de ayuno previo al pastoreo (16.5 [LS] y 2.5 [SS], h) y la presencia o no en el rumen de 12.5 kg de un material sintético indigestible. El CMS y el TP fueron mayores después de 16.5 h de ayuno y fue reducido por la presencia en el rumen de material indigestible (P<0.01). La interacción entre tiempo de ayuno y llenado previo al pastoreo sobre TP, a pesar de no haber sido significativa (P<0.06), soporta la idea de una combinación de signos, sean los responsables de modular el control de iniciación y fin de las sesiones de pastoreo. El peso de bocado no fue significativamente modificado por el tiempo de ayuno. El contenido de sólidos en el rumen y los pooles de amonia y AGV posteriores a la sesión de pastoreo, no fueron afetctados por el tiempo de ayuno previo a la sesión de pastoreo. En cambio, el ácido propiónico, fue reducido (P< 0.05) con los períodos más prolongados de ayuno. La inclusión de material inerte en el rumen previo al pastoreo, redujo significativamente (P< 0.05) los pooles de contenido total, de líquido, de MS, de MO y amonio en el rumen. Se concluyó que altos niveles de amonio y de contenido ruminal total, pueden haber estar involucrado en el control del tiempo del pastoreo en estos experimentos.

Capítulos 5 y 6. La importancia relativa de la duración del período de rebrote y la cantidad, composición química y fermentabilidad del contenido ruminal, sobre el control del TP en la primer sesión de pastoreo a.m., fueron investigadas. Cuatro vacas lecheras pastorearon individualmente parcelas de raigras (*Lolium perenne*) con 6, 9, 16, 22 y 30 días de rebrote. Los animales tuvieron acceso a la pastura hasta que voluntariamente interrumpieron la sesión de pastoreo. Previo y posterior a la sesión de pastoreo, el contenido ruminal fue evacuado, pesado, muestreado y retornado a los animales. Adicionalmente, muestras de liquido ruminal fueron tomadas inmediatamente previo a la evacuación del rumen, y aproximadamente 30, 60, 120 y 240 minutos, posteriorres a la finalización de la sesión de pastoreo. El TP no siguió ninguna tendencia específica con los días de rebrote de la pastura y exhibió profundas discontinuidades entre los días de rebrote 6 y 16. Cuando la pastura tuvo 9 días de rebrote, los animales aparentemente intentaron compensar un reducido peso de bocado y por ende TC, con un mayor TP. Los menores y mayores valores de TC y peso de bocado fueron observados en los días de rebrote 9 y 30, respectivamente. La tasa de bocado no cambió significativamente con los días de rebrote, observándose registros altos para todas las condiciones de pastoreo analizadas. Los pooles de contenido ruminal total y de MS, posteriores al pastoreo, aumentaron significativamente (P< 0.01) con los días de rebrote. Sin embargo, las vacas siempre detuvieron la sesión de pastoreo antes que la máxima capacidad del rumen fuera alcanzada. Los pooles ruminales posteriores al pastoreo, de MO y FDN se incrementaron significativamente con los días de rebrote de la pastura (P< 0.05), aunque los valores absolutos fueron bajos. El pool de AGV en rumen, incrementó linealmente (P< 0.05) con los días de rebrote de la pastura. La concentración de AGV siguió una tendencia cuadrática, con la máxima concentración observada 110 minutos después de la finalización de la sesión de pastoreo. En cambio, las fracciones nitrogenadas (N, amonio) no siguieropn ninguna tendencia definida con los días de rebrote de la pastura. En estos experimentos, ni el llenado ruminal (representado por el contenido total, MS o FDN del rumen), ni el contenido de AGV (total o de los componentes principales), amonio, pH o presión osmótica tomadas como variables individuales, estuvieron significativamente correlacionados con TP o CMS. Estas observaciones soportan la hipótesis de que más que el nivel absoluto individual de

alguno de estos componentes, la combinación de señales, debe ser la responsable de la iniciación y terminación de las comidas individuales.

Experimentos in-vitro

Capítulo 4. Se investigó la fermentabilidad de muestras de contenido ruminal, colectadas durante los experimentos de pastoreo reportados en los Capítulos 2 y 3, mediante la determinación de la producción acumulada de gas, posteriores a la incubación con inoculo proveniente de líquido ruminal de ovejas. En el Experimento 1, el contenido ruminal después de 1 h de pastoreo, exhibió mayores valores de producción acumulada de gas y menor tiempo para alcanzar la tasa de fermentación máxima ($P< 0.05$), que las vacas que pastorearon por 3.25 h. En el Experimento 2, la producción acumulada total de gas de las muestras de contenido ruminal recogidas al final de la sesión de pastoreo de las vacas LS, fue significativamente mayor ($P< 0.05$) que las vacas SS. Esta observación condice con los mayores CMS observados en las vacas LS durante el pastoreo. La precencia de material inerte en el rumen, condujo a mayor producción de gas, menor tiempo medio y mayor extensión de fermentación independientemente del período de ayuno previo. La producción total de gas previo al pastoreo, fue menor para el tratamiento LS ($P< 0.05$) que el SS. La determinación de la cinética de fermentación a través de la técnica de producción acumulada de gas, resultó adecuada para detectar cambios en el patrón de fermentación debido tanto a la remoción de contenido ruminal desde el rumen (efecto ayuno) o a cambios en el CMS durante la sesión de pastoreo.

Modelos

Capítulo 1. Un modelo de simulación dinámico y estocástico fue desarrollado con el objetivo de predecir el CMS en condiciones discontinuas de alimentación, permitiendo la combinación de hasta 5 alimentos diferentes. El modelo fue evaluado en un amplio régimen de condiciones de alimentación y mostró buena capacidad predictiva para CMS total ($R^2 = 0.95$) y de forraje ($R^2 = 0.92$), indicando buena capacidad predictiva para la substitución de forraje por concentrado. La predicción de la digestibilidad de la FDN fue menor ($R^2 = 0.65$), para un rango más reducido de condiciones de alimentación. Adicionalmente, el modelo fue utilizado para evaluar el efecto del nivel y tipo de concentrado suministrado, sobre el CMS. Las tendencias observadas estuvieron en acuerdo con los antecedentes provenientes de trabajos de investigación detallados. A pesar del comportamiento satisfactorio del modelo para predecir CMS en las condiciones que fue evaluado, presenta limitaciones en su forma actual, que inhiben una utilización más generalizada: el modelo considera el llenado del rumen como única restricción al CMS y no predice producción y absorción de los productos finales de la fermentación.

Capítulo 7. Un modelo de simulación dinámico y determinístico de digestión y absorción de nutrientes (Dijkstra et al., 1996) fue modificado y evaluado en condiciones discontinuas de alimentación. La información generada en los experimentos de pastoreo (Capítulos 2, 3, 5 y 6), fue utilizada en la evaluación del modelo. El modelo original fue modificado, para hacerlo operar bajo un régimen discontinuo de consumo de raigrás.

Los pooles de FDN y N en rumen, fueron predichos con una baja raíz del cuadrado medio del error (MSPE) de las medias observadas (12 %), para los experimentos 1 y 2 pero mayores (18 %), para el experimento 3. La raíz del MSPE fue incrementada por las observaciones correspondientes al largo período de ayuno (períodos de ayuno de hasta 20.5 h), que siguió al pastoreo en los tres experimentos. El pool de MO en rumen fue predicho con menos precisión (raíz MSPE, 16 %) que los pooles de FDN y N y requiere subsecuente validación. Los pooles y concentración de AGV en rumen, fueron predichos con una raíz de MSPE de la media observada de 32 y 33 % respectivamente, valores próximos a la variación residual observada en los experimentos. En cambio, el pool de amonia fue pobremente predicho. La representación actual de la dinámica del amonio en el modelo, requiere de modificaciones substanciales, para poder predecir producción y absorción de amonia bajo condiciones discontinuas de alimentación. Se concluye que el modelo puede ser utilizado bajo condiciones discontinuas de alimentación, para predecir digestión ruminal y absorción de nutrientes (excepto amonia) en vacas lecheras bajo pastoreo, aunque sistemas de alimentación que involucren períodos prolongados de ayuno deben ser evitados.

Conclusiones

En la Discusión General, los resultados de los ensayos en pastoreo e *in-vitro* fueron analizados en conjunto y puestos en perspectiva. Se construyó un modelo conceptual resaltando los flujos más relevantes de material e información discutidos en ésta Tesis y/o considerados relevantes en desarrollos futuros. Se concluyó que las relaciones básicas entre características de la pastura y de los animales derivadas a nivel de parche (escala reducida), pueden ser extrapoladas a nivel de parcela. Por lo tanto, la TC puede ser representada y predicha a partir del peso de bocado, que va a ser función de las características de la pastura y modulado por el nivel de llenado. La búsqueda, manipuleo y cosecha de forraje por parte de los animales, ocurren en condiciones de pastoreo, a una escala temporal muy reducida y deben ser incorporadas en el desarrollo de futuros modelos. La diferenciación entre éstos comportamientos, es un vínculo vital entre la ingestión y la digestión de forraje. Una alta TC instantánea, resulta en un mayor tamaño de partícula de la MS ingerida, con consecuencias directas sobre el volumen ruminal, digestión y desaparición de la MS desde el rumen y en consecuencia sobre el CMS. Los pooles ruminales pueden ser predichos con precisión bajo condiciones discontinuas de alimentación, a pesar de que la representación de la dinámica del N en rumen requiere mejoras substantivas. Este hallazgo es substancial, desde que la distancia actual entre los modelos de comportamiento ingestivo en pastoreo y los modelos completos de rumen, puede se acortada y el proceso indivisble de ingestión y digestión bajo pastoreo, representado adecuadamente. La pobre comprensión de los mecanismos involucrados en el control del tiempo de pastoreo, permanece como un obstáculo importante, para obtener mayores progresos en la representación y cuantificación de todo el proceso. Esta investigación ofrece información relevante acerca de la importancia relativa de varios factores probablemente involucrados en el control del TP. Adicionalmente, pone de manifiesto la necesidad de avanzar en la comprensión de la forma en la cual diferentes signos producidos en diferentes lugares, son integrados por el animal y modulan el inicio y cese de cada comida, así como otros comportamientos relevantes para el animal. En este sentido, la combinación de investigación analítica y sintética ha probado ser una estrategia efectiva.

CURRICULUM VITAE

Pablo Chilibroste is born on October 26, 1961 in Motevideo, Uruguay. He attended primary school in Villa Darwin, Soriano (School Nro 19), and at Colegio San Javier, Tacuarembó. In 1980 started studies at the Agronomy Faculty of Uruguay, where he obtained a BSc degree in agriculture. From 1984 to 1998 he worked as a private consultant for farmers and dairy Cooperatives, as a member of the technical staff of CONAGROS, SC. In 1988, he was employed by the Agronomy Faculty for teaching and research on milk production, at the Experimental Station Mario Alberto Cassinoni (EEMAC), Paysandú, Uruguay. In March 1992, he entered to the Animal Production MSc program of the Pontificia Universidad Católica de Chile, Santiago, Chile. In January 1994, obtained the Master of Science in Animal Production degree with maximum distinction. In 1995, he started a PhD program at the Wageningen Agricultural University, Institute of Animal Science, Wageningen, The Netherlands. In 1999, he won an open contest for a position as Associated Professor in Milk Production at the Agronomy Faculty, EEMAC, Paysandú, Uruguay.